Anandamide content and interaction of endocannabinoid/GABA modulatory effects in the NTS on baroreflex-evoked sympathoinhibition

Jeanne L. Seagard,1 Caron Dean,1 Sachin Patel,2 David J. Rademacher,2 Francis A. Hopp,1 William T. Schmeling,1 and Cecilia J. Hillard2
1Zablocki Department of Veterans Affairs Medical Center and Departments of Anesthesiology and Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, Wisconsin 53295
2Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, Wisconsin 53295

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CANNABINOIDS HAVE BEEN SHOWN to produce a variety of cardiovascular effects in anesthetized animals. The finding that endogenous cannabinoids (endocannabinoids), including N-arachidonylethanolamine (anandamide, AEA), and 2-arachidonylglycerol, are produced in many sites in the brain in response to neuronal depolarization raises the possibility that some cardiovascular effects of cannabinoids may be central in origin. Endocannabinoids have been found to act at neural cannabinoid (CB1) receptors to produce changes in neuronal function. Intravenous injections of Δ9-tetrahydrocannabinol (Δ9-THC), the active component of marijuana, in dogs (6, 7, 36) and cats (53) have been found to elicit prolonged depressor and bradycardic responses. Intravenous injections of AEA in rats have been found to produce similar prolonged depressor responses after brief pressor and then depressor responses (23, 25, 47, 51). The prolonged depressor responses evoked by administration of Δ9-THC and AEA have been found to be inhibited by spinal section (47, 53) and blockade of CB1 receptors by intravenous administration of the CB1 antagonist SR-141716 (25, 47). These findings suggest that the hypotension is a neurally mediated result of activation ofCB1 receptors, leading to a decrease of sympathetic activity. However, intracerebral administration of AEA in conscious rabbits was found to produce an increase in renal sympathetic nerve activity (RSNA) and a decrease in heart rate, which were blocked by SR-141716 (30), although the site of action of AEA could not be determined from the study. Brain slice studies of the rat caudal nucleus tractus solitarius (NTS), one site of termination of baroreceptor afferent fibers, showed that approximately one-half of the neurons responded to Δ9-THC or cannabinoid receptor agonists, with about two-thirds of the responding neurons increasing and one-third decreasing discharge rates (20). The alterations in discharge were blocked by SR-141716, but functions of the neurons were not known.

In other neural pathways, cannabinoids have been found to induce presynaptic and postsynaptic modulation of neuronal transmission. The responses are complex and depend on the brain region studied. Through activation of CB1 receptors, cannabinoids have been shown to presynaptically inhibit the release of glutamate and GABA in many brain regions of the rat (34). These actions are of interest for cardiovascular regulation, because glutamate has been accepted as the primary neurotransmitter released from primary baroreceptor afferent fibers in the NTS (1, 2, 4, 8), and a role for GABA modulation of baroreflex function within the NTS has also been shown (39).

Many studies suggest that cannabinoids have the potential to modulate central autonomic pathways, and this modulation could lead to altered autonomic outflows and changes in baroreflex control of blood pressure (BP). However, previous studies have generally used intravenous administration of cannabinoids, which can induce systemic responses that may mask the central nervous system effects of the drugs. The purpose of this study was to determine the role of CB1 receptor activation on baroreceptor sensory transmission within the NTS. Microinjection of AEA into the NTS was utilized to determine the...
endocannabinoid effect on baroreflex sympathetic nerve responses produced by phenylephrine (PE)-induced pressure changes in anesthetized rats. Anandamide prolonged reflex inhibition of RSNA, suggesting an increase in baroreflex sensitivity. This effect of AEA was blocked by prior microinjection of SR-141716 to block CB1 receptors in the NTS. AEA actually shortened the duration of RSNA inhibition. These results suggest that endogenous AEA is elevated during increases in BP and functions to modulate the arterial baroreflex through activation of CB1 receptors, leading to subsequent modulation of GABAergic and/or glutamatergic transmission within the NTS.

METHODS

General Methods

The protocol for the study was approved by the Animal Care and Use Committees at the Medical College of Wisconsin and the Zablocki Department of Veterans Affairs Medical Center. Sprague-Dawley rats (320–380 g) were anesthetized with pentobarbital sodium (50 mg/kg ip) with a catheter inserted into a femoral vein for supplemental administration of anesthetic and administration of PE. Arterial BP was monitored continuously from a femoral arterial cannula connected via a pressure transducer (Statham) to a polygraph (model 7, Grass Instruments) and recorded on tape (PCM recording adapter model 3000A, Vetter). A heating pad was used to maintain body temperature at 37°C.

Microinjection Studies

The head of the animal was fixed in a stereotaxic frame (Kopf), and RSNA was recorded using flexible silver wire electrodes positioned on a renal nerve exposed via a retroperitoneal approach. The electrodes were fixed in position with silicone gel (Sil Gel), allowing adjustment of the body of the animal without disturbing neural recordings. The electrophysiological signal was directed first to a high-impedance differential preamplifier (gain = 1,000, pass band = 0.1–10 kHz) and then to a filter-amplifier (gain = 400; high- and low-pass filtering = 10 Hz–3 kHz) and recorded on tape. The amplifier output was directed to a precision full-wave rectifier and low-pass (3 kHz) and recorded on tape. The electrophysiological signal was directed first to a high-impedance differential preamplifier, and the PE pressor test was repeated. After recovery of BP and RSNA, the PE pressor test was obtained as described above. Bilateral microinjection of 50 nl of SR-141716 to block CB1 receptors in the NTS sites were then performed, with care taken to ensure that the change in BP induced by PE was comparable to control for this and all ensuing tests. BP and RSNA responses were obtained for the pressor test and recovery, with time provided to allow all parameters to return to baseline. In nine rats, after recovery, 50 nl of AEA were microinjected bilaterally into the NTS sites, and the PE pressor test was repeated at 1, 3, 10, and 20 min after bilateral microinjection of AEA. To ensure that time was not a factor, a series of control PE pressor tests were performed in three additional rats, with tests repeated at 3, 10, and 20 min after the first control test.

In five separate rats, the effects of AEA were tested after prior administration of BIC. In these rats, the control response to the PE pressor test was obtained as described above. Bilateral microinjection of 50 nl of BIC was then performed at the NTS sites. After stabilization of BP and RSNA, the PE pressor test was performed. After recovery, AEA was microinjected bilaterally into the NTS sites, and the same procedure was repeated. The AEA trial was always performed within 10 min of BIC administration. To confirm that GABA blockade by BIC was effective within this period, a series of PE pressor tests were performed in four other rats at 1, 3, and 10 min after BIC microinjection. This time frame was identical to that used to test the effects of AEA after BIC microinjection.

In four additional rats, the ability of SR-141716 to block the effects of AEA was tested to determine the potential contribution of activation of CB1 receptors by AEA. In these rats, after depressor injection sites in the NTS were located, the control response to the PE pressor test was obtained as described above. Bilateral microinjections of 50 nl of SR-141716 (0.9 µM) into the NTS sites were then performed, and the PE pressor test was repeated. After recovery of BP and RSNA to baseline levels, AEA was microinjected bilaterally into the NTS as described above, and the PE pressor test was again performed at 1, 3, and 10 min after AEA administration. SR-141716 is known to have a duration of action of ≥30 min (33), and the AEA protocol was always performed within 15 min of SR-141716 administration.

At the conclusion of the protocol, the animal was killed and the medulla was removed. Frozen, transverse (40-µm) sections through the medulla were cut and examined microscopically to determine the locations of microinjections marked by blue dye, which was contained in the vehicle.
AEA Content Studies

Male Sprague-Dawley rats (200–350 g, n = 11) were anesthetized, and cannulas were inserted as described above for monitoring of BP and infusion of drugs. Seven rats were used to determine the effects of elevations in arterial BP on AEA content in the NTS, and four rats served as sham surgical or time controls. For the experimental protocol, after anesthesia and cannulation, a 30-s period of baseline BP and heart rate was recorded for each rat. For the high-BP (HBP) rats, a slow infusion of PE (0.02%, 0.2–0.4 ml/min) was initiated to elevate diastolic pressure to 50 mmHg over baseline. This rate of PE infusion was maintained for 5 min. For the control rats, all procedures except the PE infusion were performed as described for the HBP group. After the 5-min test period, the rats were immediately killed via decapitation, and an occipital craniotomy was performed to quickly remove the brain stem, which was immediately frozen by exposure to a metal plate in contact with dry ice. The region of dorsal medulla that included the NTS was then isolated from the remainder of the frozen brain stem. This region of the medulla extended from the rostral border of the area postrema to the calamus scriptorius, 1.0 mm bilateral to midline and 0.5 mm deep, to maximize inclusion of the NTS region. The NTS was dissected and stored at −80°C until the extraction procedure. Tissue was extracted according to a previously published method (32). Briefly, tissue samples were weighed and placed into borosilicate glass culture tubes containing 2 ml of acetonitrile containing 84 pmol of [2 H 8 ]AEA. Tissue was homogenized with a glass rod and sonicated on ice for 1 h. Samples were incubated overnight at −4°C to precipitate proteins. Samples were centrifuged at 1,500 g; supernatants were removed to new glass culture tubes and dried under N2. The samples were resuspended in methanol, dried under a stream of N2, resuspended in 20 μl of methanol, and stored at −80°C for liquid chromatography-mass spectrometry analysis.

AEA contents were determined by using isotope-dilution liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry. Samples (5 μl) were separated on a reverse-phase C18 column (Kromasil; 250 mm × 2 mm, 5 μm diameter) using solvent A (deionized water, 1 mM ammonium acetate, and 0.005% acetic acid) and solvent B (methanol, 1 mM ammonium acetate, and 0.005% acetic acid). Samples were eluted at 300 μl/min by a linear gradient. The percentage of solvent B increased linearly from 85% to 100% in 25 min and then was held at 100% for 10 min. A liquid chromatography-mass spectrometry detector (model SL, Agilent 1100) was used in a positive ion mode to detect [3 H 8 ]AEA and AEA by selective ion monitoring. AEA contents were normalized using wet tissue weight.

Data Analysis

Microinjection studies. For data analysis, analog-to-digital conversion of recorded parameters was performed using a computer (model 310, Hewlett-Packard, Palo Alto, CA). Arterial pressure and averaged RSNA were sampled at a frequency of 20 Hz for each control and microinjection procedure and stored on disk files for quantification and statistical analysis. Baseline values of BP and averaged RSNA were obtained using 30-s averages of each parameter sampled during the period immediately before PE baroreflex pressor tests for control and each microinjection and normalized as percentage of control baseline values. To examine the rate of recovery of RSNA after each baroreflex stimulus, RSNA was plotted vs. time, and nonlinear regression was used to fit the data to a first-order exponential recovery equation using methods described previously (33). This analysis provided values for the time constant of the rate of recovery (TCR) for RSNA. TCR is proportional to the rate at which RSNA recovers after the baroreflex-induced sympathoinhibition. For each TCR, RSNA will recover 63.2% of the remaining distance to the asymptotic value (baseline RSNA). Thus, after four TCR, RSNA will have recovered to 98% of the baseline level of RSNA. The normalized values for baseline RSNA and BP and TCR for each procedure were compared using one-way analyses of variance and differences identified using Duncan’s multiple-range test, with significance set at P < 0.05.

AEA content studies. AEA NTS content in control (normotensive) vs. HBP rats was compared using an unpaired t-test, with significance set at P < 0.05.

RESULTS

Microinjection Studies

Histological analysis of the transverse sections showed that injection sites were located bilaterally within the commissural subnuclei of the NTS (Fig. 1). Microinjection of DLH at these sites consistently produced depressor and sympathoinhibitory responses. The increases in BP produced by bolus injections of PE were reproducible and were not significantly different among all groups for the microinjection studies (range 26.7 ± 2.7 to 30.3 ± 2.2 mmHg, P = 0.87).

Microinjection of the vehicle did not result in significant changes in baseline BP or RSNA or in TCR (P = 0.313, data not shown). AEA microinjection did not produce significant changes in baseline BP or RSNA over the entire experimental period (Fig. 2). However, microinjection of AEA resulted in a significant prolongation of the reflex inhibition of RSNA as reflected by an increase in TCR (Figs. 3 and 4). At 1 min after administration, TCR was significantly increased from a control value of 17.6 ± 2.5 s to 48.4 ± 10.1 s after AEA treatment. The prolongation of TCR was no longer statistically significant 3 min after microinjection of AEA.

The role of the CB1 receptor in the effect of AEA on TCR was explored by microinjection of the CB1-selective antagonist SR-141716 into the NTS before the administration of AEA. Microinjection of SR-141716 alone or in combination with AEA did not produce any significant changes in baseline BP.
and RSNA (Fig. 5). Injection of SR-141716 before AEA administration abolished the prolongation of TCR observed in response to injection of AEA only (Fig. 6). Injection of SR-141716 alone tended to decrease TCR, but the change did not reach statistical significance.

To determine whether the prolongation of the baroreflex by AEA involved a modulation of GABA-mediated transmission, the baroreflex response to AEA was tested during blockade of postsynaptic GABA_A receptors by BIC, which would eliminate any effects due to modulation of GABA release. In support of the role of NTS GABA_A receptors in the regulation of baroreceptor responses, microinjection of BIC alone produced a significant decrease in baseline BP and RSNA compared with control (Fig. 7). Microinjection of AEA in the presence of BIC resulted in significant increases in baseline BP and RSNA back toward control level, although baseline BP remained significantly less than control. Microinjection of BIC resulted in a significant increase in TCR over control: 15.9 ± 3.2 vs. 38.9 ± 11.9 (Fig. 8). This prolongation of reflex RSNA inhibition was reversed by microinjection of AEA, which significantly shortened TCR to 14.0 ± 5.8 s at 1 min after administration. The prolongation of TCR by BIC microinjection alone was not statistically significantly different throughout a 10-min period after BIC administration (P = 0.23, data not shown), indicating...

Fig. 2. Effects of microinjection of anandamide (AEA) into the NTS on baseline levels of mean arterial blood pressure (BP) and renal sympathetic nerve activity (RSNA). Values are shown for control and for 1, 3, 10, and 20 min after AEA microinjection (AEA1, AEA3, AEA10, and AEA20). AEA did not result in significant changes in baseline BP or RSNA at any time.

Fig. 3. Effects of NTS microinjection of AEA on baroreflex inhibition and recovery of averaged RSNA in response to brief pressor responses induced by bolus injections of phenylephrine. Curve fits of RSNA recovery are shown in the bottom traces (black curves) in A and B. Compared with the control response (A), exponential fit of changes in RSNA (B), which occurred 1 min after microinjection of AEA into the NTS, shows a significant delay in recovery of RSNA to baseline levels (gray horizontal line). au, Arbitrary units.

Fig. 4. Effects of microinjection of AEA into the NTS on the time constant for the rate of the recovery (TCR) of RSNA obtained by nonlinear regression for 1, 3, 10, and 20 min after AEA microinjection. TCR represents the rate at which RSNA returns after baroreflex-induced inhibition of activity. For each TCR, RSNA will recover 63.2% of the remaining distance to the asymptotic value (baseline RSNA). Thus, after 4 TCR, RSNA will have recovered to 98% of the baseline level of RSNA. AEA significantly prolonged TCR at 1 min after microinjection of AEA, with a gradual return to control levels (Con) by 10 min. *Significantly different from all other values (P < 0.05).

Fig. 5. Effects of microinjection of SR-141716 (SR), a CB_1 receptor antagonist, and subsequent AEA microinjection into the NTS on baseline levels of mean arterial BP and RSNA. Microinjection of SR-141716 or AEA at 1, 3, and 10 min after microinjection of SR-141716 did not produce any significant changes in baseline BP or RSNA at any time.
changes in TCR. This lack of effect of AEA on TCR after prior microinjection and 10 min after SR-141716 microinjection did not produce any significant increase in TCR compared with normotensive control rats. AEA content of the NTS of HBP rats was 28.0 ± 2.0 ng/g wet tissue wt, which is equivalent to ~60 pmol/g wet tissue wt. This estimation is consistent with earlier reports of brain stem wet tissue wt, which is equivalent to 90 pmol/g brain wt (3, 13). AEA content of the NTS of HBP rats was 28.0 ± 2.0 ng/g wet tissue wt, which was a significant elevation compared with normotensive control rats (P < 0.05).

**Endogenous AEA Content in the NTS**

Because exogenous administration of AEA results in modulation of the baroreflex, it was of interest to determine whether increases in BP alter the amount of endogenous AEA within the NTS. Analysis of AEA levels showed that AEA content of the NTS of the four control rats was 21.0 ± 2.0 ng/g wet tissue wt, which is equivalent to ~60 pmol/g wet tissue wt. This estimation is consistent with earlier reports of brain stem AEA contents of 20–90 pmol/g brain wt (3, 13). AEA content of the NTS of HBP rats was 28.0 ± 2.0 ng/g wet tissue wt, which was a significant elevation compared with normotensive control rats (P < 0.05).

**DISCUSSION**

Data from this study showed that microinjection of AEA within the commissural region of the NTS resulted in an increase in the baroreflex inhibition of RSNA, reflected by a prolongation of TCR. Prolongation of TCR suggests that AEA treatment produces increased activation or disinhibition of barosensitive NTS neurons. Loss of the AEA-induced prolongation of the baroreflex response by prior microinjection of BIC into the NTS suggests that AEA primarily exerts its effect on baroreflex function through a reduction in GABA-mediated inhibition, or a disinhibition effect. However, our finding that AEA reduced TCR in the presence of GABA blockade indicates that AEA also inhibits excitatory input to the reflex, most likely via presynaptic inhibition of glutamate release. The effect of AEA was short-lived, which is not unexpected because of its rapid uptake and hydrolysis to arachidonic acid by neurons (18). It is our contention that the brief effect of AEA application indicates that the effect was highly localized and that reasonable amounts of the endocannabinoids were administered. In other words, the ability of the tissue to inactivate AEA was not overwhelmed. The loss of the effect of AEA after SR-141716 microinjection indicates the prolongation of TCR by AEA was mediated by CB1 receptors. Although not significant, SR-141716 alone produced a shortening of TCR, indicating a possible modulation of effects of endogenously released endocannabinoids. In support of a possible modulatory role of endocannabinoids in baroreflex function, we found that AEA content in the NTS was increased in rats subjected to a short period of hypertension. These data, which are the first to link AEA production to a known autonomic function, suggest that some neurons in the NTS baroreflex pathway have the potential to release AEA after depolarization, leading to modulation of reflex control of sympathetic activity and BP.

AEA has been found to exert a presynaptic inhibition of GABA release from neurons within other regions of the brain, including the hippocampus (14, 21), striatum (42), substantia nigra pars reticulata (43), periaqueductal gray (49), cerebellum (44), nucleus accumbens (27), and rostral ventromedial me-
dulla (RVLM) (50). CB1 receptors have been located on GABAergic interneurons and their nerve terminals in the human hippocampus, supporting the concept that they could play a presynaptic modulatory role in GABA release (24). AEA has also been shown to presynaptically inhibit the release of glutamate in various brain sites, including the hippocampus (15), striatum (12, 22), periaqueductal gray (49), and cerebellum (26, 44). AEA has been shown to modulate GABA and glutamate release in some of these regions in different studies. Anatomic studies examining the location of the cannabinoid neural CB1 receptor have shown that they are present in the NTS (38), located primarily on thin nerve fibers (10), supporting a presynaptic role for these receptors. Data from the present study would suggest that presynaptic inhibition of GABA release is the predominant effect of AEA in this region of the NTS of the rat. However, after GABA receptor blockade, AEA decreased the duration of baroreflex inhibition of RSNA, suggesting that AEA also acts presynaptically to inhibit the release of glutamate from baroreceptor afferent fibers but that this effect is masked by a predominant effect on GABA release.

Cannabinoid effects on sympathetic outflow and NTS neuronal activity suggest that cannabinoids could have an effect on baroreflex control of BP via an action in the central nervous system, but few studies have addressed this possible regulatory role. One early study found that a low dose of the synthetic cannabinoid 1-hydroxy-3-(1,2-dimethylheptyl)-6,6,9-trimethyl-7,8,9,10-tetrahydro-6-dibenzopyran administered to dogs resulted in a loss of the pressor reflex induced by common carotid artery occlusion (16). Because the pressor response to epinephrine was preserved in these animals, it was concluded that 1-hydroxy-3-(1,2-dimethylheptyl)-6,6,9-trimethyl-7,8,9,10-tetrahydro-6-dibenzopyran interrupted sympathetic innervation of blood vessels. In a recent study from this laboratory in anesthetized dogs, microinjection of the CB1 receptor antagonist SR-141716 into the NTS prolonged the depressor response to localized increases in carotid sinus pressure (baroreceptor stimulation), suggesting that endocannabinoids can modulate the excitability of NTS neurons involved in the baroreceptor reflex, leading to altered baroreflex regulation (33). Microinjection of the CB1 receptor agonist WIN-55212-2 into the NTS did not modulate the rate of recovery, a finding opposite to that of the present study. One possible reason for the different findings of the two studies is the level of GABAergic control in the dog vs. that in the rat. In unpublished studies, we found very little response to microinjection of GABA antagonists on the discharge of barosensitive neurons within the NTS of dogs. This lack of effect on barosensitive NTS neurons is in contrast to the inhibitory effects of GABA and GABA receptor agonists observed by Zhang and Mifflin (54) on baroreceptive neurons in rats and to the data reported here that BIC produces an increase in the baroreflex. This suggests that, in the dog, GABAergic control of NTS barosensitive neurons is not an important regulator of overall activity. Alternatively, in the dog study, localized changes in carotid sinus pressure were used to selectively activate carotid baroreceptors, whereas in the rat study, elevation of arterial pressure, which would activate all barosensitive receptors in the animal, was used. Because presynaptic inhibition of transmitter release induced by CB1 receptor activation can affect only active synapses, the differences observed in the two models could be a result of differences in the number or types of neurons activated by the different protocols.

In a study in rabbits, intracisternal administration of the CB1 receptor agonists WIN-55212-2 and CP-55940 increased baseline levels of RSNA, plasma norepinephrine concentration, and BP, effects that were attenuated by SR-141716 (30). Species differences could explain the predominant sympathoexcitatory effect of CB1 receptor activation in the rabbit compared with our study in the rat, but the difference could also be due, in part, to the route of administration used to deliver the cannabinoid agonists. Intracisternal administration will result in the exposure of many regions of the brain not included in this study, which could alter sympathetic activity. In a study by Varga et al. (48), intravenous administration of AEA in rats produced an initial brief increase in discharge of barosensitive, presynaptic neurons in the RVLM, which preceded transient increases in RSNA and BP. This initial burst of neuronal activity was followed by a more prolonged hypotension and sustained increases in sympathetic and RVLM neuronal activities, possibly due to baroreflex-evoked changes in response to the hypotension. This study supports the hypothesis that AEA can have varied effects on sympathetic activity, depending on the areas of the brain exposed to the cannabinoid.

We found that microinjection of BIC alone into the NTS produced a significant decrease in BP and RSNA, suggesting a tonic GABAA receptor modulation of baroreceptive neurons in the NTS. This is supported by other studies that have identified a GABAA modulatory role in the NTS on BP. Studies have found that microinjection of GABA or muscimol, a GABAA receptor agonist, into the NTS produced hypertension and tachycardia (5, 31, 39) as well as an increase in RSNA (41), which were blocked by injection of BIC. Microinjection of BIC alone has been found to produce a decrease in BP and heart rate (5, 31) and RSNA (31). However, some studies have not found any change in baseline BP in response to NTS microinjection of GABA (45). There is also evidence of a role for GABA receptors in the tonic regulation of BP (5, 11), which may be greater under certain conditions than the contribution of GABAA receptors. The differences in responses to administration of GABA, GABA agonists, and GABA antagonists among the studies are not clear but may include dose, sites of injection, or level of anesthesia in the animals. Furthermore, a critical factor appears to be the need to microinject GABA, agonists, or antagonists bilaterally to prevent compensatory responses from being evoked from the contralateral, unaffected NTS (39).

We also found that microinjection of BIC prolonged the baroreflex inhibition of RSNA, similar to other studies that found a role for GABA-mediated effects on baroreflex function. NTS microinjections of muscimol and baclofen, a GABAB receptor agonist, have been found to block depressor and bradycardic responses produced by stimulation of the aortic depressor nerve in rats (40), suggesting a role for GABAA and GABAB receptor modulation of baroreflex function. This attenuation of reflex responses is reflected by attenuation of discharge of baroreceptive neurons by GABA and agonists. Microinjection of GABA into the NTS in rats has been found to attenuate the activity of neurons evoked by carotid sinus nerve stimulation (28). This effect of GABA was antagonized by BIC, which increased activity of the evoked neurons when administered alone. Exposure to GABA,
GABA\textsubscript{A} and GABA\textsubscript{B} agonists has also been found to attenuate the discharge of NTS neurons evoked by aortic nerve stimulation (54). Iontophoretic administration of GABA and muscimol inhibited spontaneous discharge and blocked the excitation of second-order baroreceptor NTS neurons to aortic depressor nerve stimulation. Similar effects were observed in response to administration of a GABA\textsubscript{B} receptor agonist, baclofen. The sites of action of muscimol and GABA were identified to be postsynaptic, whereas baclofen was found to have a presynaptic inhibitory effect. Earlier studies have not commented on any prolongation of a baroreflex response due to administration of BIC. The prolongation in the inhibition of RSNA observed in the present study in response to BIC suggests that decreased GABA modulation of barosensitive NTS neuronal activity can lead to an increase in baroreflex sensitivity and duration. The similar reflex prolongation by AEA, which was eliminated by BIC, suggests that AEA acts via attenuation of GABA release.

Using a sensitive, quantitative mass spectral assay, we have found that the content of the endocannabinoid AEA is significantly increased within the NTS after a brief but intense period of hypertension induced by PE administration. This finding, to our knowledge, is the first demonstration of a modulation of brain endocannabinoid content in response to changes in sensory input produced by physiological changes in a known parameter, i.e., BP. There is no evidence that AEA is stored in neurons, so changes in AEA content in a tissue or cell likely reflect changes in releasable or released AEA (18). The increase in AEA content could result from an increase in synthesis or a decrease in inactivation. Two mechanisms of AEA inactivation have been described: 1) an AEA cellular reuptake process followed by 2) hydrolysis of AEA by fatty acid amide hydrolase (19). Neither of these processes exhibits mechanisms of regulation that would be dependent on neuronal activity (19, 46). In contrast, AEA is synthesized by neurons from phospholipid precursors in response to changes in neuronal activity (37), so our present hypothesis is that the increase in AEA content in the NTS is due to increased neuronal activity, which results in increased AEA synthesis.

In summary, microinjection of AEA into the NTS prolonged baroreflex-induced inhibition of RSNA in the rat. This effect was due to activation of CB\textsubscript{1} receptors and appears to involve presynaptic modulation of GABA release from nerve endings in the NTS. Blockade of GABA\textsubscript{A} receptors eliminated the “sympathoinhibitory” effect of AEA and suggested a possible role for AEA in the presynaptic inhibition of glutamate. An increase in AEA content in the NTS in response to a period of brief hypertension suggests that barosensitive input evokes increases in endogenously released AEA, which can in turn modulate the baroreflex.

**Perspectives**

The possible dual modulatory effect of AEA in the presynaptic modulation of glutamate vs. GABA release could result in differential modulation of the baroreflex; the direction of the modulation would depend on the level of GABAergic tone. When NTS GABAergic tone is high, CB\textsubscript{1} receptor activation would result in a potentiation of the baroreflex and a reduction in sympathetic drive. One condition in which an upregulation of GABAergic tone occurs in the NTS is the hypertension found in spontaneously hypertensive rats (SHR) and surgically induced rat models (52), which also have attenuated baroreflex sensitivity (17). In support of this hypothesis, $\Delta^9$-THC (23, 29) and AEA (25) have been shown to reduce BP to normotensive levels in SHR and in rats with surgically induced hypertension. AEA was more effective in lowering BP in anesthetized and conscious SHR than in control rats; this finding further supports the hypothesis that the effectiveness of CB\textsubscript{1} receptor activation is dependent on the level of preexisting sympathetic tone, which is dependent on central GABAergic tone. Although the level of GABAergic tone in the NTS of humans is not known, THC exposure results in orthostatic intolerance, marked by an increase in heart rate and a decrease in BP. Although the hypotension is thought to be due primarily to inhibition of norepinephrine release from nerve terminals innervating vascular smooth muscle, the increase in heart rate may be a baroreflex response to the hypotension made possible by withdrawal of parasympathetic tone and, thus, not dependent on increases in effective sympathetic drive, which could be blunted by a mechanism similar to that seen at vessels. Although it is tempting to speculate that the effects on the baroreflex seen in this study play a role in the hypotensive effects of THC in humans, the question cannot be answered at this time because of complications from peripheral effects of THC. Studies that have restricted cannabinoid exposure to selected central sites have not been done in humans. In addition, baroreflex responses to increases in BP are needed to quantitate effects of activation, and not unloading, of baroreceptors. Also, there may be a difference between acute and chronic exposure to cannabinoids. In an early study in primates, chronic exposure to THC resulted in an increase in baroreceptor gain to a pressor response and an augmentation of parasympathetic vs. an attenuation of sympathetic control of heart rate (9). Changes in sympathetic activity appeared to adapt over an extended exposure. Finally, the data presented here indicate that cannabinoid-induced changes in baroreflex function may be dependent on the extent of GABAergic tone, and the amount of tonic GABAergic drive to the NTS and other brain regions in humans is not known. The potential differential alteration in parasympathetic vs. sympathetic drive may be of critical importance, but more work in carefully controlled studies in humans is needed. Nonetheless, activation of CB\textsubscript{1} receptors directly could be of therapeutic benefit for the treatment of essential hypertension in humans. However, because hypertension results in an elevation of AEA content in the NTS, a better therapeutic approach could be the use of AEA reuptake or fatty acid amide hydrolase inhibitors to prolong the action of AEA and enhance endogenous CB\textsubscript{1} receptor activation. Although we have not examined AEA inactivation in this region directly, the short effective half-life of exogenous AEA in NTS suggests that the processes for inactivation would be good therapeutic targets to enhance the action of the endogenous CB\textsubscript{1} system. This could result in localized CB\textsubscript{1} receptor activation without the psychoactive side effects that would result from administration of a direct-acting CB\textsubscript{1} receptor agonist.

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