HIV-1-Tat excites cardiac parasympathetic neurons of nucleus ambiguus and triggers prolonged bradycardia in conscious rats

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HIV-1-Tat excites cardiac parasympathetic neurons of nucleus ambiguus and triggers prolonged bradycardia in conscious rats. Am J Physiol Regul Integr Comp Physiol 306: R814–R822, 2014. First published April 2, 2014; doi:10.1152/ajpregu.00529.2013.—The mechanisms of autonomic imbalance and subsequent cardiovascular manifestations in HIV-1-infected patients are poorly understood. We report here that HIV-1 transactivator of transcription (Tat, fragment 1–86) produced a concentration-dependent increase in cytosolic Ca2+ in cardiac-projecting parasympathetic neurons of nucleus ambiguus retrogradely labeled with rhodamine. Using store-specific pharmacological agents, we identified several mechanisms of the Tat-induced Ca2+ elevation: 1) lysosomal Ca2+ mobilization, 2) Ca2+ release via inositol 1,4,5-trisphosphate-sensitive endoplasmic reticulum pools, and 3) Ca2+ influx via transient receptor potential vanilloid type 2 (TRPV2) channels. Activation of TRPV2, nonselective cation channels, induced a robust and prolonged neuronal membrane depolarization, thus triggering an additional P/Q-mediated Ca2+ entry. In vivo microinjection studies indicate a dose-dependent, prolonged bradyarrhythmic effect of Tat administration into the nucleus ambiguus of conscious rats, in which neuronal TRPV2 played a major role. Our results support previous studies, indicating that Tat promotes bradycardia and, consequently, may be involved in the QT interval prolongation reported in HIV-infected patients. In the context of an overall HIV-dependent autonomic dysfunction, these Tat-mediated mechanisms may account for the higher prevalence of sudden cardiac death in HIV-1-infected patients compared with general population with similar risk factors. Our results may be particularly relevant in view of the recent findings that significant Tat levels can still be identified in the cerebrospinal fluid of HIV-infected patients due to efficient antiretroviral therapy.

Peripheral neuropathy has been denoted as a putative cause of parasympathetic deregulation because of its frequent association with dysautonomia in HIV (48). The HIV invades the brain soon after systemic infection, targeting microglia and perivascular macrophages, while replicating in astrocytes (41). Whereas neurons are resistant to HIV infection, the infected brain cells may release viral components and neurotoxins that alter neuronal function (41). Antiretroviral therapy is efficient in reducing the viral load, but cannot prevent production of early viral proteins (50). Since the alteration of the sympathovagal balance occurs early in HIV infection and evolves with disease progression (4, 40), the early HIV proteins may contribute to autonomic impairment.

Among the early viral components, the transactivator of transcription (Tat) protein is an important factor in the HIV-induced pathogenesis of AIDS, contributing to HIV-associated neurological and cardiovascular impairment (27, 49). Tat is expressed at high levels in the brains of HIV-1-infected individuals and has been reported to alter neuronal function (35). Moreover, Tat has recently been identified in the cerebrospinal fluid of patients with suppressed viremia due to efficient antiretroviral treatment (32). Transgenic mice expressing Tat in the myocardium present with bradycardia (22). The first 72 residues of Tat, encoded by the first exon, are critically involved in Tat neuronal effects (35). In this study, we investigate the effect of a widely used Tat fragment (1–86) (35), referred to as Tat, on cardiac preganglionic neurons of nucleus ambiguus, which are key regulators of the parasympathetic cardiac tone (38).

MATERIALS AND METHODS

Ethical approval. Animal protocols were approved by the Institutional Animal Care and Use Committee from Thomas Jefferson University and Temple University. All efforts were made to minimize the number of animals used and their suffering.

Chemicals. All chemicals were from Sigma-Aldrich (St. Louis, MO), unless otherwise mentioned. Tat protein (1–86) was from Prospect (East Brunswick, NJ). HIV-1 Tat was heat-inactivated by repeated (10 times) heating (75°C for 30 s) and cooling (4°C for 1 min), as previously reported (10).

Animals. Neonatal and adult Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were used in this study. Neonatal (1–2 days old) rats of either sex were used for retrograde tracing and neuronal culture, and adult male rats (250–300 g) were used for cardiovascular measurements.

Neuronal labeling and culture. Preganglionic cardiac vagal neurons of nucleus ambiguus were retrogradely labeled by intrapericaridal injection of rhodamine [X-rhodamine-5-(and-6)-isothiocyanate;
5(6)-XRTC], 40 μl, 0.01% (Invitrogen, Carlsbad, CA), as reported by Brailoiu et al. (8, 9, 12). Medullary neurons were dissociated and cultured 24 h after rhodamine injection, as previously described (8, 9, 12). In brief, the brains were quickly removed and immersed in ice-cold Hanks’ balanced salt solution (HBSS; Mediatech, Manassas, VA). Neonate rats were euthanized by decapitation. The ventral side of the medulla (containing nucleus ambiguus) was dissected and minced, and the cells were subjected to enzymatic and mechanical dissociation. Cells were filtered using a sterile 40-μm filter/cell strainer (Falcon, Fisher Scientific, Waltham, MA) and were plated on glass coverslips in Neurobasal-A medium (Invitrogen) containing 1% GlutaMax (Invitrogen), 2% antibiotic-antimycotic (Mediatech, Herndon, VA), and 10% FBS. Cultures were maintained at 37°C in a humidified atmosphere with 5% CO₂. Cytosine β-arabino furanoside (1 μM) was added to the culture to inhibit glial cell proliferation (52).

**Calcium imaging.** Measurements of intracellular Ca²⁺ concentration, [Ca²⁺], were performed as previously described (8, 9, 12). Briefly, cells were incubated with 5 μM Fura-2 AM (Invitrogen) in HBSS at room temperature for 45 min and washed with dye-free HBSS. Coverslips were mounted in an open bath chamber (RP-40LP, Warner Instruments, Hamden, CT) on the stage of an inverted microscope Nikon Eclipse TI-E (Nikon, Melville, NY), equipped with a Perfect Focus System and a Photometrics CoolSnap HQ2 charge-coupled device camera (Photometrics, Tucson, AZ). During the experiments, the Perfect Focus System was activated. Fura-2 AM fluorescence (emission 510 nm), following alternate excitation at 340 and 380 nm, was acquired at a frequency of 0.25 Hz. Images were acquired and analyzed using NIS-Elements AR 3.1 software (Nikon). After appropriate calibration with ionomycin and CaCl₂ and Ca²⁺ free and EGTA, respectively, the ratio of the fluorescence signals (340/380 nm) was converted to Ca²⁺ concentrations (28).

**Measurement of membrane potential.** The relative changes of neuronal membrane potential were evaluated using bis-(1,3-dibutylbarbituric acid)-trimethine-oxonol, DiBAC₄(3), a voltage-sensitive dye, as reported (9, 12). Neurons were incubated for 30 min in HBSS containing 0.5 mM DiBAC₄(3), and the fluorescence was monitored at 0.17 Hz (excitation/emission 480 nm/540 nm). Calibration of DiBAC₄(3) fluorescence was performed using the Na⁺ - K⁺ ionophore gramicidin in Na⁺ -free physiological solution and various concentrations of K⁺ and N-methylglucamine, as described previously (13).

**Surgical procedures.** Adult male Sprague-Dawley rats were anesthetized with a mixture of ketamine hydrochloride (100–150 mg/kg) and acepromazine maleate (0.2 mg/kg), as reported previously (5, 9, 12). Animals were placed into a stereotaxic instrument: a guide C315G cannula (PlasticsOne, Roanoke, VA) was bilaterally inserted into the nucleus ambiguus. The stereotaxic coordinates for identification of nucleus ambiguus were 12.24 mm posterior to bregma, 2.1 mm from midline, and 8.2 mm ventral to the dura mater. A C315DC cannula dummy (PlasticsOne) was used to prevent contamination. For transmitter implantation, a 2-cm-long incision was made along the linea alba. A calibrated transmitter (E-mitters, Series 4000; Mini Mitter, Sunriver, OR) was inserted into the intraperitoneal space, as previously described (9, 12). Subsequently, the abdominal musculature and dermis were sutured individually, and animals returned to individual cages.

**Telemetric heart rate monitoring.** The signal generated by transmitters was collected via series 4000 receivers (Mini Mitter, Sunriver, OR), as previously described (9, 12). VitalView software (Mini Mitter) was used for data acquisition. Each data point represents the average of heart rate per 30 s.

**Noninvasive blood pressure measurement.** In rats with cannula inserted into the nucleus ambiguus, blood pressure was noninvasively measured using a volume pressure recording sensor and an occlusion tail-cuff (CODA System, Kent Scientific, Torrington, CT), as described previously (12). One week after the insertion of the cannula, rats were exposed to handling and training every day for 1 wk. The maximum occlusion pressure was 200 mmHg, minimum pressure was 30 mmHg, and deflation time was 10 s. Two measurements were done per 30 s (one cycle), and the average was used to calculate heart rate and systolic, diastolic, and mean arterial blood pressure. Ten acclimatization cycles were done before starting the experiments.

**Microinjection into nucleus ambiguus.** One week after surgery (telemetric studies) or after another week of training (tail-cuff measurements), bilateral microinjections into the nucleus ambiguus were carried out using the C3151 internal cannula (33 gauge, PlasticsOne) and a Neuros Hamilton syringe, model no. 7000.5 KH SYR, without animal handling. In the tail-cuff method, trained rats were in the animal holder for the duration of the experiment. For recovery, at least 2 h were allowed between two injections. Injection of 1-glutamate (5 mM, 50 nl) was used for the functional identification of nucleus ambiguus (8, 9).

**Statistical analysis.** Data were expressed as means ± SE. One-way ANOVA followed by post hoc analysis using Bonferroni and Tukey tests was used to evaluate significant differences between groups. P < 0.05 was considered statistically significant.

**RESULTS**

**HIV-1 Tat increases cytosolic Ca²⁺ concentration in cardiac preganglionic neurons of nucleus ambiguous.** In rhodamine-labeled neurons, Tat (500 nM) induced a fast increase in intracellular Ca²⁺ concentration, [Ca²⁺], followed by a rapid decrease and a plateau maintained at half maximal level (Fig. 1A). Heat-inactivated Tat (500 nM) had negligible effects on [Ca²⁺], of cardiac vagal neurons (Δ[Ca²⁺] = 14 ± 2.1; Fig. 1, A and B). Tat (5 nM, 50 nM, 500 nM, and 5,000 nM) elevated [Ca²⁺], with a mean amplitude of 39 ± 1.8 nM, 319 ± 3.4 nM, 647 ± 6.3 nM, and 762 ± 8.3 nM, respectively (Fig. 1B), and a calculated EC₅₀ of 66 nM. Six cells were examined in each treatment group. Statistical significance was achieved for the latter three concentrations of Tat. Characteristic examples of changes in 340 nm/380 nm Fura-2 AM fluorescence ratio of rhodamine-labeled neurons in response to Tat (500 nM) and heat-inactivated Tat (500 nM) are shown in Fig. 1, C and D. In vitro systems test Tat effects at concentrations varying between 100 and 500 nM (1, 21, 46, 56). Since robust responses were elicited with 500 nM Tat, similar to previous reports (1, 21, 56), we used this concentration in our following experiments. Tat elicits Ca²⁺ influx in cardiac vagal neurons. We used a pharmacological approach to test the involvement of several Ca²⁺-permeable ion channels to Tat-induced Ca²⁺ increase in vagal neurons. Inhibition of N-type Ca²⁺ channels with ω-conotoxin GVIA (100 nM, 20 min) did not significantly modify the shape of the Ca²⁺ signal produced by Tat (500 nM) in rhodamine-labeled preganglionic neurons (Fig. 2A). The amplitude and the area under curve were similarly unaffected: the amplitude of the Tat-induced response was 647 ± 6.3 nM (n = 6) in the absence vs. 638 ± 7.3 nM (n = 6) in the presence of N-type Ca²⁺ channel blocker (Fig. 2B); the areas under curve were 2,463 ± 41 nM × min and 2,397 ± 54 nM × min, respectively (Fig. 2B). Pretreatment of neurons with ω-conotoxin MVIIIC (100 nM, 20 min), a blocker of P/Q-type of Ca²⁺ channels, slightly reduced the amplitude of the Tat-dependent Ca²⁺ elevation (Δ[Ca²⁺] = 541 ± 5.7 nM; n = 6), and significantly diminished the area under the curve, which measured 1,603 ± 27 nM × min in this case (Fig. 2B). Since transient receptor potential vanilloid 2 (TRPV₂) is a Ca²⁺-permeable nonselective cation channel expressed in the nucleus ambiguus (34), we tested the effect of TRPV₂ inhibitor tranilast (44, 45). In the presence of tranilast (100 μM, 20 min),
Tat elicited a significantly blunted Ca\(^{2+}\) response, with a lower peak and shorter-duration plateau (Fig. 2A); the amplitude measured \(411 \pm 5.1\) nM \((n = 6)\), and the area under the curve was \(680 \pm 19\) nM \(\times\) min (Fig. 2B). When both TRPV2 and P/Q channels were inhibited, Tat no longer elicited a plateau phase, and the initial transient increase in [Ca\(^{2+}\)]\(_i\) was additionally reduced (Fig. 2A). \(\Delta[Ca^{2+}]_i\) was \(359 \pm 4.7\) nM \((n = 6)\), and the area under the curve was \(447 \pm 17\) nM \(\times\) min (Fig. 2B). The functional presence of Ca\(^{2+}\)-permeable TRPV2 in rhodamine-labeled cardiac vagal neurons was detected by the Ca\(^{2+}\) response produced by application of TRPV2 agonist probenecid (100 \(\mu\)M) (2). The response to probenecid was abolished in Ca\(^{2+}\)-free saline or by the TRPV2 blocker tranilast (100 \(\mu\)M, 20 min) (Fig. 3, A and B).

Tat releases Ca\(^{2+}\) from endoplasmic reticulum and lysosomal Ca\(^{2+}\) stores. In absence of extracellular Ca\(^{2+}\), Tat (500 nM) triggered a fast and transient [Ca\(^{2+}\)]\(_i\) elevation (Fig. 4A) by \(376 \pm 4.2\) nM \((n = 6)\) at the peak of the response (Fig. 4B). This response was significantly reduced in amplitude compared with the effect of Tat in Ca\(^{2+}\)-containing saline. Thapsigargin (1 \(\mu\)M), an inhibitor of the sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\) ATPase, drastically decreased the effect of Tat in Ca\(^{2+}\)-free saline; \(\Delta[Ca^{2+}]_i\) was \(79 \pm 2.4\) nM, \(n = 6\) neurons (Fig. 4, A and B). Blocking ryanodine receptors with ryanodine...
(10 μM, 1 h) did not significantly interfere with the response to Tat (Δ[Ca^{2+}]) was 371 ± 39 nM; n = 6). In the presence of xestospongin C (10 μM, 15 min), a blocker of inositol 1,4,5-trisphosphate (IP3) receptors (IP3R), rhodamine-labeled cardiac vagal neurons responded to Tat with a greatly diminished increase in [Ca^{2+}], by 81 ± 1.8 nM (n = 6) at the peak of the response (Fig. 4, A and B). A significant reduction in the Tat-mediated response was also observed in the presence of bafilomycin A1 (1 μM, 1 h), a V-type ATPase that inhibits lysosomal acidification (6); Δ[Ca^{2+}] was 176 ± 3.7 nM, n = 6 (Fig. 4, A and B). Pretreatment with both bafilomycin and xestospongin C largely abolished the Ca^{2+} increase produced by Tat (Δ[Ca^{2+}]) was 16 ± 2.3 nM; n = 6 (Fig. 4, A and B).

**Tat produces a concentration-dependent depolarization of cardiac vagal neurons.** Tat (500 nM) robustly depolarized cardiac-projecting parasympathetic neurons retrogradely labeled with rhodamine, an effect that lasted for the duration of the experiment (Fig. 5A). Conversely, heat-inactivated Tat (500 nM) produced negligible effects on membrane potential (Δvoltage was 0.83 ± 0.1 mV, Fig. 5, A and B). Increasing concentrations of Tat (5 nM, 50 nM, 500 nM, and 5,000 nM) induced neuronal depolarizations with amplitudes of 0.6 ± 0.4 mV, 4.7 ± 0.6 mV, 11.9 ± 0.8 mV, and 14.3 ± 1.1 mV, respectively (Fig. 5B), and a calculated EC_{50} of 101 nM. The responses to the latter three concentrations of Tat were significant (n = 6 neurons per each condition; P < 0.00001).

**Tat-induced depolarization involves TRPV2 activation.** Treatment of the neurons with BAPTA-AM (200 μM, 20 min), a fast Ca^{2+} chelator, markedly reduced the amplitude and the duration of Tat-induced neuronal depolarization (Fig. 6A). In neurons pretreated with the TRPV2 inhibitor tranilast (100 μM, 20 min; Fig. 6A), Tat produced a similar, strongly diminished response. The amplitude of the depolarization elicited by Tat (500 nM) was reduced from 11.9 ± 0.8 mV to 4.2 ± 0.7 mV in the presence of BAPTA and to 4.1 ± 0.8 mV in the presence of tranilast, while the area under the curve decreased from 73.1 ± 5.4 mV × min to 7.68 ± 0.41 mV × min and to 6.65 ± 0.39 mV × min, respectively (Fig. 6B).

**Microinjection of Tat into the nucleus ambiguus produces bradycardia in conscious rats.** In conscious, freely moving rats bearing cannulas implanted into the nucleus ambiguus, microinjection of control saline (50 nl) produced negligible effects on heart rate, monitored telemetrically. Microinjection of l-glutamate (5 mM, 50 nl) elicited bradycardia, without a change in blood pressure (Fig. 7A), indicating the correct placement of the cannula into the nucleus ambiguus (8, 9, 17). Two hours after l-glutamate administration, microinjection of Tat (500 fmol/50 nl) triggered a sharp and sustained decrease
Fig. 5. Tat depolarizes cultured cardiac preganglionic neurons of nucleus ambiguus. A: representative changes in membrane potential elicited by Tat (500 nM) or heat-inactivated Tat (500 nM) in rhodamine-labeled neurons. B: concentration-response curve indicating the effect of Tat (5–5,000 nM; \( EC_{50} = 101 \) nM) and heat-inactivated Tat (500 nM) on the resting membrane potential of cardiac vagal neurons; \( P < 0.000001 \) compared with resting membrane potential (*) or to the effect of 500 nM Tat (#).

in heart rate (Fig. 7A). Similar responses were recorded using the tail-cuff method (Fig. 7A). The tail-cuff method allowed measurement of blood pressure; as shown in Fig. 7A, microinjection of Tat into the nucleus ambiguus had no effect on blood pressure. Heat inactivation of Tat prevented its bradycardic effects (Fig. 7A). Tat (5 fmol, 50 fmol, and 500 fmol/50 nl) decreased the heart rate of conscious rats by 6 \( \pm \) 1.7 beats per min (bpm), 26 \( \pm \) 8 bpm, and 68 \( \pm \) 2.1 bpm, respectively (Fig. 7B). Only the latter two effects were statistically significant (\( P < 0.000001 \)). Administration of heat-inactivated Tat (500 fmol/50 nl) did not result in a significant change in heart rate (4 \( \pm \) 0.7 bpm). Five animals were used per each treatment group. In vivo studies evaluating Tat effects in the brain use Tat at a concentration of 3 or 4 \( \mu \)g/\( \mu \)l, which is >1,000 times higher than that commonly used in vitro (36, 60). Similarly, microinjection in the rat nucleus ambiguus of higher concentrations of Tat (5–500 fmol/50 nl, i.e., 0.1–10 \( \mu \)M) elicited the bradycardic responses. The explanation may reside in the fact that Tat may be locally diluted upon microinjection (60) or that Tat is not only sensitive to oxidation, but also is particularly sticky, able to bind even to silanized glass surfaces (as in the syringe barrel and needle used in stereotaxic injections) (36).

Measuring actual levels of Tat in the extracellular space in the central nervous system has proven difficult because of the lack of successful ELISA strategies and because native Tat is incredibly sensitive to oxidation. Nanomolar levels of Tat have been detected in the sera of HIV-infected patients, but these values may be underestimated, since Tat can be trapped by heparan sulfate expressed on the cell surfaces (46). However, patients with suppressed viremia due to effective antiretroviral treatment still present nanomolar levels of Tat in the cerebrospinal fluid (32). This concentration is expected to be higher near productively infected cells. As such, although Tat levels in situ (i.e., in the brain extracellular space) remain to be determined, it is conceivable that levels sufficient to promote neuronal activation may well be achieved near HIV-1-infected glia cells.

**Tat-induced bradycardia is mediated by TRPV2.** Microinjection of TRPV2 antagonist tranilast (100 \( \mu \)M, 50 nl) alone produced a negligible increase in heart rate (Fig. 8A), with a mean amplitude of 9 \( \pm \) 0.7 bpm (\( n = 5 \)) and with an area under the curve of 36 \( \pm \) 4.1 bpm \( \times \) min (Fig. 8B). Concomitant microinjection of tranilast and Tat (500 fmol/50 nl) drastically reduced the amplitude and duration of Tat-induced bradycardic effect (Fig. 8A). In the presence of tranilast, Tat decreased the heart rate by 53 \( \pm \) 2.9 bpm (\( n = 5 \)), compared with a decrease by 68 \( \pm \) 2.1 bpm produced by Tat alone (\( n = 5 \) rats). The areas under the curve were more dramatically affected: 52 \( \pm \) 4.3 bpm \( \times \) min after combined tranilast and Tat administration, compared with 432 \( \pm \) 8.3 bpm \( \times \) min produced by Tat alone (Fig. 8B).

**DISCUSSION**

HIV-1 Tat is a 72(86)-101(104)-amino acid regulatory protein, essential for viral transcription and replication; the first 56 residues of Tat are well conserved, indicating important functional roles (20, 49). The cysteine-rich region (residues 22–37)

![Fig. 6. Tat-induced depolarization in Ca\(^{2+}\)-dependent and TRPV2-mediated. A: representative traces depicting Tat (500 nM)-induced depolarizations in the absence (black) and presence of Ca\(^{2+}\) chelator BAPTA (light gray) or of TRPV2 blocker tranilast (Tran; dark gray). B: comparison of the amplitudes (top) and areas under curve (AUC; bottom) of Tat-triggered depolarizations in the conditions mentioned in A; *\( P < 0.00001 \) compared with Tat.](image-url)
and the basic domain (residues 31–61) play major roles in Tat-induced neurotoxic and excitatory effects (35). Upon release by infected microglia, macrophages, and astrocytes, Tat targets uninfected neurons to trigger Ca\(^{2+}\) responses and depolarization (7, 10, 35). These mechanisms have been implicated in both Tat-mediated neuronal responses and HIV-induced neurotoxicity (43, 46, 49).

The first observation of the present study is that Tat (fragment 1–86) produced a robust increase in [Ca\(^{2+}\)]i of cardiac vagal neurons, characterized by an initial transitory peak with high amplitude, followed by a long-lasting, smaller plateau. Next, we identified that the sustained phase of the Tat-induced effect on [Ca\(^{2+}\)]i, was mostly dependent on Ca\(^{2+}\) entry via TRPV2 nonselective cation channels and only partially mediated by P/Q-type of voltage-activated Ca\(^{2+}\) channels. We and others reported a major role of P/Q-type of Ca\(^{2+}\) current in the physiology of cardiac parasympathetic ambiguous neurons (9, 11, 12, 31). A Tat-mediated P/Q Ca\(^{2+}\) current has also been described in suprachiasmatic nucleus neurons (19). Nonetheless, not much is known about the TRPV2 function in nucleus ambiguous neurons of nucleus ambiguus depolarized in response to Tat application, and the effect was concentration-dependent. The depolarization occurred rapidly and presented a sustained plateau that lasted for the entire duration of the experiments. Ca\(^{2+}\) chelation or TRPV2 inhibition produced likewise reductions of the initial amplitude and abolished the sustained phase of the depolarization. The residual Tat-mediated depolarization had largely identical kinetics and amplitudes in the presence of BAPTA-AM or tranilast pretreatment, supporting a correlation of the Ca\(^{2+}\) increase and TRPV2 stimulation activation in the mechanism. A putative explanation of the residual depolarization observed with Tat in the presence of BAPTA may lie in the ability of Tat to depolarize the neuronal cell membrane when applied extracellularly to outside-out membrane patches,
Cardiovascular complications of antiretroviral treatment have been described (33, 61, 63). Particularly, patients receiving antiretroviral drugs may be at increased risk for myocardial infarction (33, 61). However, recent studies indicate that cardiovascular risk status, as estimated by Framingham risk score categories (&lt;6% and &gt;=6%), remained relatively stable over 144-wk period in patients enrolled in ARIES (Atazanavir, Ritonavir, Induction with Epzicom Study; EPZ108859) (62). ARIES was a large-phase IIIb/IV treatment-simplification clinical trial in which antiretroviral therapy-naive subjects who achieved virologic suppression by week 30 on a regimen of abacavir/lamivudine plus atazanavir/ritonavir were randomized to remain on their original regimen or discontinue the ritonavir component of the regimen for up to a total of 144 wk (53–55). The effects of antiretroviral therapy on QT interval prolongation (14, 24) or on dysautonomia in HIV (14) were found insignificant. Conversely, greater QT interval duration has been reported in HIV-infected patients with asymptomatic autonomic neuropathy compared with HIV-infected patients without neurological involvement (59), indicating that dysautonomia in HIV infection has a negative impact on cardiac function. Moreover, HIV-infected individuals are at a significantly higher risk for sudden cardiac death compared with the general population with similar risk factors (58). Lethal arrhythmias account for as much as 20% of total sudden cardiac deaths in HIV-1-infected patients and are six times more prevalent in these patients than in the control population (58). Of the sudden cardiac death victims with recent laboratory investigations, over half had undetectable viral loads (58); nonetheless, patients under controlled antiretroviral therapy showing viral load suppression both in the blood and cerebrospinal fluid still present significant levels of Tat in the cerebrospinal fluid (32).

**Perspectives and Significance**

HIV-1 transactivator of transcription, or Tat, has several pleiotropic functions in addition to its transcriptional activity (27). We report here that Tat produces a sustained bradycardia by targeting neurons of nucleus ambiguus that modulate the cardiac vagal tone. We characterize the underlying cellular mechanisms of Tat-induced activation of nucleus ambiguus neurons. Our findings provide a novel mechanism for the
cardiac autonomic dysfunction reported in HIV-infected patients (4, 18, 26, 40).

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

AUTHOR CONTRIBUTIONS

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