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COMMENTARY

Endothelin and bradykinin: 'brothers-in-arms' in Chagas vasculopathies?

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Reports of Chagas disease are increasing in non-endemic populations across the globe. Apart from vector eradication and prevention efforts by public health organizations, current pharmacological interventions are sparse and show important side effects. In this issue of the *BJP*, Andrade *et al.* elegantly demonstrate a new pharmacological paradigm whereby *Trypanosoma cruzi* host cell invasion requires significant cross-talk between receptors for kinins and endothelins. It is shown, for example, that acting via both ET_A and ET_B receptors, endothelin-1 (ET-1) cooperates with the (TLR2/CXCR2/B_2 kinin receptor) complex to activate inflammatory processes in response to invading trypomastigotes.

This study by Andrade *et al.* prompts, however, several important questions, summarized in this Commentary, such as the putative role of chymase-dependent production of ET-1, the contentious protective role of ACE inhibitors in Chagasic patients, the unexplored role of *de novo* formed B_1 receptors for kinins triggered by cytokines and the putative role of compartmentalized calcium pools in host cell invasion by trypomastigotes.

LINKED ARTICLE
This article is a commentary on Andrade *et al.*, pp. 1333–1347 of this issue. To view this paper visit http://dx.doi.org/10.1111/j.1476-5381.2011.01609.x

Abbreviations
BK, Bradykinin; ET-1, endothelin-1; TLR, Toll-like receptor

Introduction

More than 15 million people worldwide are infected with Chagas disease and 14 000 new cases are reported yearly (http://www.dndi.org/press-releases/2010/694-wha-chagas-resolution.html). Since April 2010, the United States Food and Drug Administration has approved new blood donor tests to screen blood, tissue and organ donors for the blood-borne Chagas parasite *Trypanosoma cruzi* (http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2010/ucm210429.htm). The European Commission on Health and Research has also recently recognized that new drugs and drug targets (in particular for chronic infections), vaccines, epidemiology, diagnostics and vector control against *T. cruzi*, currently all constitute key research gaps to be bridged (http://ec.europa.eu/research/health/infectious-diseases/neglected-diseases/pdf/nid-leaflet_en.pdf).

Whether the increase in awareness on *T. cruzi* is prompted by migration, induced by global warming, of the South American infectious parasite to more Northern niches (i.e. to putatively 'non-endemic' countries) or by *T. cruzi*-infected travellers (Tanowitz *et al.*, 2011), there are intensified international efforts towards a better understanding of Chagas disease, transmitted mainly by its principal vector, the triatomine bug (or 'kissing' or *reduviid* bug).

Roles of endothelin and bradykinin receptors in Chagas vasculopathies

A decade ago, Salomone *et al.* (2001) first reported increased plasma levels of endothelin-1 (ET-1) in Chagas patients. Furthermore, others have suggested that bradykinin (BK), an

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activator of inflammation in Chagas disease, may be of causal importance to the severity of the disease (Mukherjee *et al.*, 2003).

The paper by Andrade *et al* in this issue of *BJP* (Andrade *et al.*, 2012) addresses novel mechanisms of the possible pharmacological control of Chagas disease in the cardiovascular system. In a series of *in vitro* and *in vivo* assays, the authors elegantly show that endogenous ET-1 interacts with the inflammatory cascade driven by the Toll-like receptor TLR2, the chemokine receptor CXCR2 and the B_2 kinin receptor, which is triggered by invading *T. cruzi* trypomastigotes. These authors also report that HOE-140 a B_2 kinin receptor antagonist, reduces ET-1 induced increases of *T. cruzi* invasion in human smooth muscle cells.

It is striking that two of the most potent vasoactive peptides reported to date, the vasodilator BK and the vasoconstrictor ET-1, are shown in this paper to play an important role in interstitial oedema occurring in *T. cruzi*-infected hamsters, as well as in the invading properties of the trypomastigote form of the parasite in endothelial, smooth muscle and cardiac cells. The ability of both peptides to induce microcirculatory plasma leakage, independently of *T. cruzi* infection, is well documented.

Andrade *et al*. (2012) also suggest that the pro-inflammatory and invading properties of *T. cruzi*, involving kinin and/or ET receptors, share common intracellular signalling pathways, as concomitant treatments with BQ-123/BQ-788 (ET_A/ET_B receptor antagonists) and HOE 140 (B_2 receptor antagonist) fail to result in additive antagonism in the *in vitro* and *in vivo* models explored in this paper.

T. cruzi invasion, on the other hand, is reduced by the metabolically resistant pseudo peptide HOE 140, a potent B_2 receptor antagonist. It is intriguing that no important roles for the inducible B_1 receptors for kinins were reported by Andrade *et al*. (2012) in the inflammatory conditions associated with *T. cruzi* invasion of host cells. Yet Scharfstein *et al*. (2000) had previously disclosed that *T. cruzi* trypomastigotes produce cruzipains, proteases which may act as kallikrein-like zymogens, that enable generation not only of BK from kininogens, but probably of des-Arg^9-BK as well. Worthy of notice, however, the same group (Todorov *et al.*, 2003) did show that under lipolysaccharide-induced inflammatory conditions, *T. cruzi* invasion of human umbilical vein endothelial cells was significantly reversed by the B_1 antagonist [Leu^8]des-Arg^9-BK.

To explain, on the other hand, the contribution of endogenous ET-1 in the progression of this parasitic disease, Andrade *et al*. (2012) argue in favour of a mobilization of endothelin-sensitive, tissue mast cells attracted by the trypomastigotes released from ruptured pseudocytes. Mast cell infiltration has been reported in myocardium of *T. cruzi*-infected patients (Cabral *et al.*, 2002). Noteworthy, chymase is also significantly involved in the production of ET-1 (1–31) and ET-1 *in vivo* (Simard *et al.*, 2009). Whether the increased plasma ET-1 levels associated with *T. cruzi* infection are the consequence of activation of mast cell chymase is an interesting question to be addressed.

Another intriguing question prompted by the study of Andrade *et al*. (2012) is whether Chagas disease patients should be treated with ACE inhibitors. Indeed, Andrade and colleagues performed an interesting *in vitro* experiment in which B_2 receptors of *T. cruzi*-infected human umbilical vein endothelial cells were rescued by treating them with the non-thiol-containing ACE inhibitor, lisinopril, thus allowing an enhanced B_2 receptor-dependent penetration of the parasite into these human endothelial cells. These *in vitro* results would argue for a deleterious effect of ACE inhibitors in *T. cruzi* patients. This suggestion, however, needs to be validated in animal models *in vivo* and eventually in patients.

In support of this note of caution, results of a recent study by de Paula Costa *et al* (2010) favoured the opposing hypothesis. That study showed that repeated treatment of acutely *T. cruzi*-infected mice with enalapril, another non-thiol-containing ACE inhibitor which is used in Chagasic patients to ameliorate heart functional capacity and its remodelling, reduced serum levels of pro-inflammatory cytokines and infiltration of mononuclear cells into the heart, while increasing the number of cardiac mast cells. It is possible that these alterations could lead to limitation of cardiac fibrosis in the chronic phase of Chagas disease. De Paula Costa *et al*. (2010), however, attribute the cardio-protective properties of enalapril in Chagasic patients to a reduction of angiotensin II synthesis, a pathway not contemplated by Andrade *et al*. (2012) in their study.

Perspectives

Upon access to the host's circulation, infective *T. cruzi* trypomastigotes invade cells to form cysts, where they are less susceptible to detection and immunological attack. After escaping into the cell's cytoplasm, these transform into the amastigote form to replicate repeatedly and differentiate into the mammalian-stage trypomastigotes, which are released into the blood stream after the host cell's death to infect new cells (Mortara *et al.*, 2008). It would be interesting to assess if antagonism of ET or B_2 kinin receptors also affect re-colonization of host cells by *T. cruzi* by actions at the stage of dividing amastigotes.

The recent development of highly specific monoclonal antibodies to label *T. cruzi* epitopes (Taniwaki *et al.*, 2007) should also facilitate considerably studies to characterize the peri- and intracellular migration of trypomastigotes in host cells, using 3-D confocal microscopy, in real-time conditions. These new studies would extend the pioneering work of Mortara *et al*. (1999), who demonstrated, for the first time, the usefulness of confocal microscopy to assess the severity of *T. cruzi* infestation within host cells. These types of studies should also allow the assessment of the contribution of high intracellular calcium containing organelles in the parasitic invading capacities of *T. cruzi*.

Indeed. Andrade *et al*. (2012) show, for example, that *T. cruzi* invasion of host cells is sensitive to inhibition by verapamil, but not thapsigargin. Furthermore, it is now established that reticular formations are involved importantly in nuclear/cytosolic calcium exchanges (Avedanian *et al.*, 2011). Could these specialized intracellular structures play an important role in the invasive capacity of *T. cruzi* in host cells?

It is also of interest that B_1 and B_2 as well as ET_A and ET_B receptors were identified at the nuclear membranes of human vascular endothelial and smooth muscle cells as well as in cardiomyocytes (El-Bizri *et al.*, 2003; Bkaily *et al.*, 2011).

Whether these nuclear receptors are activated following *T. cruzi* penetration is an intriguing concept to be explored further.

Finally, studies on genetic susceptibility of the host to infection by Chagas disease remain few and sparse even when considering the variation in transmission rates reported in different endemic populations, as recently reviewed by Tibayrenc (2010). Could host resistance to *T. cruzi* invasion or reduced severity of Chagas disease in some populations be related to variation in innate immune responses?

Conclusion

The significant cross-talk between endothelin ET_A/ET_B and B_2 kinin receptors (and thus between endogenous endothelins and kinins) in *T. cruzi* invasion of various host cell types has been reported here by Andrade *et al.*, (2012). The concepts conveyed in this paper prompt new questions for pharmacologists and cellular biologists interested in tackling the mechanistic pathways triggered by these two GPCR-stimulating peptides in the course of the highly debilitating Chagas disease. Such mechanistic studies also need to be complemented by data on population genetics to assess the future impact of pharmacogenomics in targeting endemic and non-endemic populations affected by this parasitic disease.

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