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Impact of acute and chronic stressor experiences on heart atrial and brain natriuretic peptides in response to a subsequent stressor

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ABSTRACT

The impact of stressful events on processes related to cardiovascular functioning might vary with previous stressor experiences, just as such sensitization effects have been detected with respect to several neurochemical and hormonal processes. The present investigation assessed the impact of a psychosocial stressor on factors directly or indirectly related to cardiovascular functioning among CD-1 mice that had previously experienced an acute or chronic stressor regimen. These factors included plasma variations of atrial and brain natriuretic peptides (ANP and BNP, respectively), inflammatory cytokines in plasma, mRNA expression of natriuretic peptides and inflammatory cytokines in the ventricles, and norepinephrine (NA) levels and utilization within the locus coeruleus, a brain region implicated in cardiac functioning. A social stressor (exposure to a dominant mouse) increased NE levels and utilization within the locus coeruleus, plasma corticosterone, cytokine and ANP levels. Among mice initially exposed to an acute stressor (restraint), NE utilization, ventricular ANP mRNA expression, and plasma interleukin-6 (IL-6) concentrations were markedly increased by the subsequent social stressor. In chronically stressed mice some of the effects of the social stressor were dampened, including changes of plasma corticosterone, locus coeruleus NE utilization, as well as plasma and ventricular IL-6 mRNA expression. Conversely, plasma ANP was markedly enhanced by the combined stressor events as was ventricular BNP and IL-1β mRNA expression. It seems that stressors may profoundly influence (sensitize or desensitize) on factors that could influence cardiovascular functioning. It remains to be determined whether these actions would be translated as pathophysiological outcomes.

Introduction

Stressor experiences have been associated with increased depression and cardiovascular disturbances, and comorbidity between these pathologies has frequently been reported (Lesperance and Frasure-Smith, 2000). Numerous processes could be involved in mediating the effects of stressors on the development of cardiovascular disturbances, including peripheral and central nervous system mechanisms. For instance, stressor-provoked effects on norepinephrine (NE) neurons of the locus coeruleus may influence cardiac receptors and increase heart rate, blood pressure and ventricular contraction rate, thereby influencing vulnerability to tachycardia or ventricular fibrillation (Dalack and Roose, 1990). Furthermore, baroreceptors located in the aortic arch and carotid sinus, which control heart rate and blood pressure, can be influenced by stressors and may contribute to cardiac events such as ventricular arrhythmia (Hatton et al., 1997). Indeed, acute stressors were found to reduce the sensitivity of the baroreflex or to reset it to a higher pressure (Hatton et al., 1997; Porter, 2000) and to increase coronary vasoconstriction, heart rate and blood pressure (Dalack and Roose, 1990).

Atrial and brain natriuretic peptides (ANP and BNP, respectively) are cardiac hormones that have been implicated as markers of cardiac diseases, including heart failure, cardiac hypertrophy, arrhythmia and left ventricular dysfunction (Goetze et al., 2004; Mekontso-Dessap and Brochard, 2006). Ordinarily, ANP is synthesized in the atria and ventricular heart, whereas BNP is mainly produced by the ventricular heart. These hormones promote augmented glomerulal filtration and excretion of excessive sodium in urine, normalization of blood pressure, and the reduction of heart NE activity (Piechota et al., 2008). The rise of tachycardia and blood pressure was associated with the release of ANP, and both ANP and BNP were elevated in association with myocardial dysfunction (Grabie et al., 2003; Tavener and Kubes, 2006). Consistent with the view that stressful experiences may be a risk factor for cardiovascular disease, it was reported that emotional stressors and chronic insults increased heart gene expression of natriuretic peptides coupled with increased ANP release (Ueyama et al., 2003; Wann et al., 2010). This does not necessarily suggest that these peptides contribute to the pathology, and indeed, their function may also be one of protecting the heart (Ichihara et al., 2009; McGrath and de Bold, 2005).

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In addition to natriuretic peptides, cardiovascular diseases have been associated with inflammatory factors (Campbell and MacQueen, 2004; Carpeggiani et al., 2004; Lesperance and Frasure-Smith, 2000). For instance, stressors were shown to increase circulating levels of pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α (Anisman et al., 2008; Frazure-Smith et al., 2009), which might contribute to altered cardiac functioning (Libetta et al., 2007). These pro-inflammatory cytokines can stimulate BNP synthesis (Campbell and MacQueen, 2004), and it also appears that ANP may be a modulator of immune activity and cytokine functioning (Blumenthal et al., 2005). In addition, both stressors and bacterial endotoxin (lipopolysaccharide) challenges increased ANP concentrations in plasma and in the left ventricle, and increased left atrial and ventricular IL-1β and TNF-α mRNA expression (Wann et al., 2010), and the stressor and endotoxin treatment synergistically increased plasma ANP (Wann et al., 2010).

Stressful events have been linked to heart disease, and natriuretic peptides could potentially serve as markers of cardiac dysfunction. However, the data concerning stressor effects on these peptides, as well as on processes that might be tied to them (e.g., cytokines), is limited. Moreover, most studies have been concerned with the immediate effects of stressors on ANP and BNP, and have not focused on the relatively protracted effects that may occur. In this regard, it is known that stressor-induced brain neurochemical changes, and some aspects of hypothalamic-pituitary-adrenal (HPA) functioning, may be subject to a sensitization effect, wherein re-exposure to stressors days or weeks following an initial insult, may promote much greater neurotransmitter or neuroendocrine changes than would ordinarily be elicited by the proximal stressor itself (Anisman and Merali, 2003; Hayley et al., 1999).

Given the links between natriuretic peptides, cytokines and locus coeruleus NE activity, in relation to cardiac functioning, it was of interest in the present investigation to assess (a) whether an acute stressor vs. a chronic stressor regimen (comprising variable challenges over 21 days) would have a protracted effect on NE activity within the locus coeruleus, inflammatory factors (cytokines) and ANP and BNP (in plasma and heart), and (b) whether these initial stressor experiences would influence the impact of a subsequent challenge (social disturbance) on these same processes.

Methods

Subjects

Male CD-1 mice (N = 92) were obtained from Charles River Canada (St. Constant, Quebec) at 6 weeks of age. They were housed in groups of four upon arrival and allowed to acclimatize to the laboratory setting for 10–14 days in a temperature-controlled vivarium with lights on from 0800 h to 2000 h and food and water freely available. All procedures were conducted in accordance with the guidelines set out by the Canadian Council on Animal Care and were approved by the Carleton University Animal Care Committee.

Stressor procedures

Table 1 provides a description of the sequence of events for each of the treatment conditions. As well, the table indicates the number of mice in each of the groups; however, as will be described shortly, since the number of samples varied in some assays (e.g., plasma cytokines), the df varied in the statistical analyses for the different outcome measures.

After the acclimatization period, mice were housed individually and randomly assigned to either a chronic, acute, or no stressor condition. Chronically stressed mice were exposed to a procedure previously shown to elicit depressive-like behaviors and to alter brain monoamine activity (Anisman et al., 2007; Tannenbaum et al., 2002). Specifically, they were exposed to a series of different stressors on each of 21 days (applied twice daily) with a different stressor applied in the morning and afternoon. The stressors comprised the following: physical restraint in semicircular Plexiglas tubes (4 cm diameter × 12 cm long) with tails taped to prevent mice from turning (15 min); exposure to predator odor (rat) by placing the mouse in a cage containing soiled rat bedding (60 min); placing the mouse in a cage containing bedding that was soaked with water (60 min); forced swim in water of 20 °C within a plastic cylinder of 30 cm diameter and 27 cm high (5 min). The animals were returned to their individual home-cages between the two stressor sessions of each day. On the morning of the last day (day 21) of the chronic stressor regimen mice were exposed to a single stressor session that comprised placing them in a tight fitting triangular baggie (with a hole for the nose) resulting in complete restraint (15 min).

Mice in the Acute stressor condition experienced the "baggie stressor" (placement in a tight fitting triangular baggie for 15 min) on a single occasion. This permitted direct comparison between those mice that received only the 'baggie stressor' on this single occasion and those that had received a series of different challenges prior to the baggie stressor. This particular stressor was selected for the acute condition as it provides complete restraint, and we have found this procedure to be particularly potent given the pronounced neuroendocrine and brain neurochemical changes that are provoked.

After the baggie (restraint) stressor session, mice in the chronic and the acute stressor conditions remained in their individual home-cages for the ensuing 21 days without disturbance other than routine changes of bedding. The third group, which comprised mice in the No stressor condition, remained undisturbed in their home-cages over the 42-day period prior to decapitation (i.e., paralleling the 21 days of the initial stressor phase and 21 days of the stressor-free period in the

Table 1
Experimental design and sequence of treatment procedures.

Stressor conditions	Stressor phase (21 days)		Resting phase (21 days)	Test day
	Days 1–20	Day 21		
No stressor	No stressor	No stressor	No stressor	No stressor (n=15/group) Social stressor (n=16/group)
Acute stressor	No stressor	Restraint	No stressor	No stressor (n=15/group) Social stressor (n=15/group)
Chronic stressor	Multiple stressors	Restraint	No stressor	No stressor (n=15/group) Social stressor (n=16/group)