Neurohormonal activation in canine degenerative mitral valve disease: implications on pathophysiology and treatment

Neurohormonal systems play a critical role in canine degenerative mitral valve disease (DMVD). DMVD results in mitral regurgitation, which reduces forward cardiac output and increases intracardiac pressures. These changes trigger neurohormonal responses that ultimately result in maladaptive cardiac remodelling, congestion and heightened morbidity and mortality. Medical therapies such as ACE inhibitors and spironolactone derive their benefit by interrupting or suppressing these neurohormonal responses. Thus, knowledge of neurohormonal mechanisms can lead to a better understanding of how to treat DMVD.

M. A. Oyama
DOI: 10.1111/j.1748-5827.2009.00801.x
Accepted: 19 June 2009
Conflicts of Interest: MAO has acted as a paid consultant to IDEXX Laboratories and has received funding for research referenced in this work.

DMVD decreases forward cardiac output and increases intracardiac hydrostatic pressure. These changes elicit the response of multiple neurohormonal systems, whose activation maintains adequate cardiac output, blood pressure and tissue perfusion. The preservation of blood flow and pressure is accomplished by increasing renal sodium and water retention and eliciting peripheral vasoconstriction. The sympathetic nervous system (SNS) and the renin-angiotensin-aldosterone system (RAAS) are two well-described systems that are central to these effects. Fluid retention and vasoconstriction are furthered by the activation of the arginine vasopressin (AVP) and endothelin-1 (ET-1) systems. The natriuretic peptide system provides an endogenous counterbalance to these effects by promoting diuresis and vasodilation; however, in dogs with advanced DMVD, the effects of the natriuretic peptides are overwhelmed and the balance of activity favours vasoconstriction and fluid retention, resulting in increased cardiac afterload, deleterious myocardial remodelling and congestive heart failure (Fig 1) (Ware and others 1990, Pedersen and others 1995, Marcondes and others 2006).

All neurohormonal systems have developed in a similar manner. There is an “input” afferent arm that detects alterations in physiological parameters such as pressure, oxygen tension or sodium concentration, and an “output” efferent arm that uses various neurohormonal molecules and targets receptors to modulate physiological responses (Table 1). The presence or absence of these receptors on different tissue types confers specificity to the system. Thus, actions can be targeted to myocardial cells, vascular smooth muscle or specific portions of the nephron. A system’s time course of activation is also important. Theoretically, therapy that disrupts these systems should be prescribed at the exact time point they become maladaptive. If therapy is prescribed before this point, it may be ineffective, incur financial waste and put the patient at risk for adverse side effects without hope of counterbalancing benefit. The remainder of this review will discuss the characteristics of the most important neurohormonal systems, their time course of activation, and what ramifications these characteristics have on deciding when and how to treat DMVD.

The input arm of the SNS consists of pressure and chemical receptors within the central nervous system, carotid sinus, aortic arch, renal afferent arteries and heart. Reduced cardiac output and arterial hypotension offloads pressure receptors, resulting in a centrally mediated decrease in vagal tone and increase in sympathetic tone. Central and peripheral chemoreceptors respond to changes in lactic acid and oxygen and carbon dioxide tension. Hypercapnia, hypoxia and acidosis result in heightened sympathetic tone. When stimulated, SNS efferent activity is achieved through increased firing of...
sympathetic nerve terminals and release of norepinephrine (NE), decreased NE reuptake, increased central NE turnover and increased adrenal medullary production of epinephrine. These effector molecules bind to adrenergic receptors primarily in the heart and vasculature.

In the normal heart, the primary adrenergic receptor is the $\beta_1$ receptor. Binding of NE triggers a cascade of secondary messengers including cyclic adenosine monophosphate and protein kinases. Protein kinases phosphorylate a wide assortment of regulatory molecules within the myocar-
dial cell that increases intracellular calcium, resulting in increased force of contraction and increased heart rate. In peripheral smooth muscle, the primary adrenergic receptor is the $\alpha_1$ receptor. Binding of NE results in increased intracellular calcium that elicits vasoconstriction. Thus, increased SNS activity at the level of the heart and vasculature supports cardiac output and blood pressure, and the classic “fight or flight” role of the SNS is fulfilled. In the setting of heart disease, where cardiac injury is chronic and progressive, elevation of SNS activity is persistent and maladaptive. Chronically elevated SNS tone contributes to acceleration of disease through multiple processes, including myocyte hypertrophy, persistent tachycardia, increased myocardial oxygen demand, increased afterload, receptor downregulation, inefficient energy production and loss of myocytes through apoptosis and necrosis (Opie 2002). Thus, the harmful effects of long-term SNS activation outweigh the short-term beneficial effects, and suppression of SNS activity is a cornerstone of successful medical therapy in human beings with heart failure. In both human beings (Davila and others 2005) and dogs with experimental mitral regurgitation (Hankes and others 2006), SNS activity is increased relatively early in disease, first locally at the level of the heart and kidneys, and then in a more generalised systemic manner as disease progresses. This early local activity is mediated by NE release from SNS nerve endings within the heart and increases NE concentrations in the myocardial interstitial fluid and coronary sinus blood in dogs with experimentally produced mitral regurgitation (Farrell and others 2001, Hankes and others 2006). As disease progresses, local NE spills over into the general circulation, resulting in elevated plasma NE concentration. In dogs with DMVD, circulating NE levels tend to parallel the development of heart enlargement and are consistently elevated once congestive heart failure is present (Ware and others 1990, Table 1. Major cardiovascular effects of various neurohormonal systems

<table>
<thead>
<tr>
<th>System</th>
<th>Input sensors</th>
<th>Output molecules</th>
<th>Target organs</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sympathetic nervous system</td>
<td>Baroreceptors and chemoreceptors*</td>
<td>Norepinephrine and epinephrine</td>
<td>Vascular smooth muscle</td>
<td>Vasodistension</td>
</tr>
<tr>
<td>Renin-angiotensin-aldosterone system</td>
<td>Baroreceptors and chemoreceptors†</td>
<td>Angiotensin II</td>
<td>Vascular smooth muscle</td>
<td>Vasodistension</td>
</tr>
<tr>
<td>Natriuretic peptide system</td>
<td>Myocardial stretch</td>
<td>Atrial natriuretic peptide and B-type natriuretic peptide</td>
<td>Kidney</td>
<td>Hypertrophy, diuresis</td>
</tr>
<tr>
<td>Arginine vasopressin system</td>
<td>Osmoreceptors Baroreceptors</td>
<td>Arginine vasopressin</td>
<td>Vascular smooth muscle</td>
<td>Hypertrophy</td>
</tr>
<tr>
<td>Endothelin-1</td>
<td>Endothelial cells</td>
<td>Endothelin-1</td>
<td>Vascular smooth muscle</td>
<td>Vasodistension (ET-A)</td>
</tr>
</tbody>
</table>

*Central nervous system, carotid sinus, aortic arch, renal afferent arteries and heart.†Juxtaglomerular cells and macula densa.
Neurohormonal activation in DMVD

Uechi and others 2002, Santos and others 2006). In human beings, plasma NE and epinephrine levels are strongly correlated to survival (Cohn and others 1984, Kaye and others 1995). In virtually all instances of human systolic heart failure (reduced contractility) beta-blocker therapy is recommended (Hunt and others 2005). Why then are beta-blockers not routinely prescribed to dogs or people with DMVD? One of the primary indications for beta-blockade, namely reduced contractility, is difficult to demonstrate in dogs with DMVD. Routine echocardiographic measures of contractility such as fractional shortening or ejection fraction are confounded by the presence of moderate to severe mitral regurgitation. Both diastolic volume overload and low resistance to left ventricular ejection result in ventricular wall motion that appears “hyperdynamic”, and fractional shortening values are often normal or even exceed the reference range (Fig 2). Other echocardiographic indices such as absolute or indexed end-systolic diameter may be a better method to assess contractility in dogs with DMVD (Borgarelli and others 2007). In dogs with advanced DMVD, administration of beta-blockers can result in acute decompensation, hypotension and congestive heart failure. Thus, despite compelling evidence in both human beings and experimental models indicating that chronic SNS stimulation is harmful, specific guidelines regarding routine beta-blockade in dogs with DMVD are lacking until more clinical data are available.

Renin-angiotensin-aldosterone system

The input arm of the RAAS consists of the juxtaglomerular cells of the afferent renal arteriole and the macula densa cells of the distal convoluted tubule. Either decreased renal blood flow or renal tubular sodium chloride concentration elicits production of preprorenin from the juxtaglomerular cells. Preprorenin is quickly cleaved to prorenin and then to renin by a trypsin-like enzyme. Renin converts angiotensinogen that is produced by the liver into angiotensin I. Angiotensin I is then converted into angiotensin II (ATII) by angiotensin converting enzyme (ACE) as it passes through the pulmonary capillaries (Fig 3). The biological actions of ATII are contributory to the progression of heart disease and elevated ATII levels are predictive of cardiovascular death (Roig and others 2000). The output arm of the RAAS system involves two different ATII receptors, AT-R1 and AT-R2. The heart and peripheral vascular smooth muscle are rich in AT-R1, and binding increases contractility, vasoconstriction, hypertrophy, remodelling and myocardial fibrosis. AT-R2 are also present in the kidneys and activation promotes active sodium exchange within the proximal and distal convoluted tubules, vasoconstriction of renal blood vessels and passive retention of sodium within the loop of Henle. Centrally located AT-R, mediate increased thirst while AT-R within the cortex of the adrenal gland stimulate aldosterone secretion. Thus, the net effect of ATII and AT-R binding is fluid retention, vasoconstriction and vascular and myocardial remodelling. The functions of AT-R are generally contrary to those of AT-R, in that AT-R elicits vasodilation; however, selective stimulation of AT-R can also induce myocyte damage, hypertrophy and cell death (Henrion and others 2001).

The RAAS is generally stimulated in dogs with congestive heart failure secondary to DMVD (Knowlen and others 1983, Sisson 2004); however, this is not uniform across all studies (Häggström and others 1997). In both human beings and dogs, the time point of systemic activation approximates the development of symptomatic disease; however due to differences in measurement techniques, breed, dietary sodium intake and concurrent medications, it is difficult to know exactly when during the course of disease the RAAS is activated. In human beings,
RAAS activation is preceded by SNS activation (Francis 1990); however, data specific to human beings with primary mitral valve disease are lacking. In dogs with mild asymptomatic DMVD, renin, angiotensin I, ATII and aldosterone are either not elevated (Häggström and others 1997, Fujii and others 2007) or variably elevated (Pedersen and others 1995, 1999, Pedersen 1996, Rush and others 2000) in comparison with normal dogs. ACE inhibition improves survival in dogs with symptomatic DMVD (COVE Study Group 1995, Ettinger and others 1998) but does not appear to substantially reduce risk for congestive heart failure when used in dogs with asymptomatic disease (Kvart and others 2002, Atkins and others 2007). These findings are most consistent with a relatively late time course of systemic RAAS activation.

Interestingly, components of the RAAS are found in many tissues including the heart and kidney, and local tissue RAAS may be of importance. Fujii and others (2007) reported that myocardial ACE activity was increased in dogs with mild experimental mitral regurgitation while circulating renin, angiotensin I, ATII and aldosterone were normal. Thus, similar to the SNS, local RAAS activity may be important in the early stages of DMVD prompting consideration of ACE inhibitors with high tissue-ACE specificity (as opposed to systemic ACE); however, it should be noted that overzealous ACE inhibition may be detrimental. In dogs with experimental mitral regurgitation, aggressive ACE inhibition suppresses myocardial collagen formation and leads to progressive cardiac chamber enlargement (Dell’Italia and others 1997).

Finally, alternate pathways of ATII production may be able to circumvent ACE. In dogs, this alternate system involves chymase and kallikrein (Dell’Italia and others 1995, Sasaguri and others 1999, Fujii and others 2007). Tissue chymase converts angiotensin I to ATII, while kallikrein converts angiotensinogen directly to ATII. Thus, both ATII and aldosterone can be elevated in (human) patients despite the use of ACE inhibitors. Tang and others (2002) reported that 35 and 85 per cent of human beings receiving ACE inhibitors demonstrated elevated serum aldosterone and ATII concentrations, respectively. Due to this phenomenon of “aldosterone escape”, adjunctive therapy with specific aldosterone blockers, such as spironolactone, or specific AT-R, blockers is attractive (Tang and Francis 2005).

**NATRIURETIC PEPTIDES**

The natriuretic peptide system consists of atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). In human beings, cardiac production and release of ANP and BNP are primarily mediated through stretch of the myocardial tissue (Magga and others 1998), although other stimuli such as SNS or RAAS activity (Wiese and others 2000, Sakata and others 2009), ET-1 (Rademaker and others 2004), ischaemia (Goetze and others 2003) and inflammation (Vila and others 2008) can also trigger release. Both peptides are released in the form of a pro-hormone which is rapidly cleaved to an inactive N-terminal product (NT-proANP, NT-proBNP) and a biologically active C-terminal end (C-ANP, C-BNP). C-ANP and C-BNP primarily bind to natriuretic peptide receptor-A (NPR-A), which is located in the heart, kidney, vascular smooth muscle, brain and adrenal glands. Binding induces cyclic guanine monophosphate production, which causes vasodilation, increased glomerular blood flow and filtration rate, reduced sodium uptake, natriuresis and diuresis. In this manner, the natriuretic peptide system acts as the heart’s “volume sensor”, such that an increase in blood volume triggers natriuresis and diuresis. Both C-ANP and C-BNP have relatively short half-lives and are best...
suited for short-term modulation of volume. Both molecules are degraded by circulating endopeptidases and both bind to NPR-C, which internalises and hydrolyses the molecule. NPR-C is distributed mainly in areas of high blood flow such as the kidney, adrenal gland, pulmonary tissue, heart and brain. Both ANP and BNP are foetal genes that are predominantly expressed at birth, and then reinduced in instances of disease. Both peptides are produced by atrial tissue; however, in instances of disease, ventricular BNP production is increased. CNP is predominantly produced in the brain and vascular tissues where it is thought to act in a paracrine manner to cause vasodilation.

ANP and BNP are increased in dogs with heart failure and help reduce diastolic volume and improve diastolic function (Häggström and others 1994, 1997, 2000, Asano and others 1999, Lainchbury and others 2000, Boswood and others 2003, 2008, Greco and others 2003, MacDonald and others 2003, DeFrancesco and others 2007, Oyama and others 2008). In dogs with DMVD, the natriuretic peptides are correlated to heart size and clinical signs (Swedberg and others 2005, Maisel and others 2000, MacDonald and others 2000, MacDonald and others 2003, Oyama and others 2008, Tarnow and others 2009). The time course of activation generally mirrors the development of cardiac enlargement. In dogs with mild disease, ANP (Asano and others 1999) and BNP (MacDonald and others 2003, Oyama and others 2008) are only variably elevated. The sensitivity of NT-proANP may be greater than NT-proBNP for detection of mild disease (Asano and others 1999, Häggström and others 2000). As disease progresses, ANP and BNP tend to increase, and are significantly elevated before the onset of congestive heart failure (Fig 4) (Tarnow and others 2009). In human beings with valve disease, ANP and BNP typically, but not consistently, correlate to severity of regurgitation, heart size and symptoms (Sutton and others 2003, Mayer and others 2004, Detaint and others 2005, Ray 2006). In one study (Detaint and others 2005), BNP independently predicted mortality over a 4-year follow-up period. Greco and others (2003) reported that in a cohort of 23 dogs, C-ANP greater than 95 pg/ml was associated with shorter median survival, and MacDonald and others (2003) reported that in a cohort of 25 dogs with DMVD, for every 10 pg/ml increase in C-BNP mortality over 4 months’ time increased by 44 per cent.

The practical considerations of natriuretic peptide activation involve using natriuretic peptides either as therapeutic agents or as markers of disease severity and outcome. In human beings with acute heart failure, infusion of BNP is associated with both haemodynamic and symptomatic improvement. Most (Publication Committee for the VMAC Investigators 2002, Keating and Goa 2003, Peacock and others 2005, Sakr and others 2008) but not all (Miller and others 2008) studies demonstrate that addition of BNP infusion to routine care reduces length of hospital stay and incidence of future hospitalisation. The value of BNP infusion in dogs with DMVD is unknown. In human beings (Silver and others 2004, Arnold and others 2007) and dogs (Boswood and others 2008), NT-proANP and NT-proBNP have been used to assist in diagnosis of heart disease, staging of disease severity and discrimination between cardiac and non-cardiac causes of dyspnea (Fine and others 2008, Oyama and others 2008). In human beings, treatment decisions based on natriuretic peptide levels yielded better outcomes (Troughton and others 2008, Sakr and others 2009). However, this finding has recently been called into question (Pfisterer and others 2009). The use of natriuretic peptide levels to predict onset of congestive heart failure, guide treatment and predict outcome in dogs with DMVD are intriguing applications that await further study.

**FIG 4.** Circulating concentrations of N-terminal B-type natriuretic peptide (NT-proBNP) and left atrial to aortic root ratio (LA:Ao) in dogs with DMVD. Note the progressive increase in both NT-proBNP and left atrial size as disease progresses from minimal disease to overt congestive heart failure. Graph adapted from Tarnow and others (2009)

**ARGININE VASOPRESSIN**

The input arm of the AVP system involves both osmotic and non-osmotic stimuli,
Osmoreceptors in the portal veins and hypothalamus monitor plasma osmolality and increase central AVP release from the posterior pituitary. Non-osmotic regulation via baroreceptors in the heart, great vessels and carotid sinus also mediates AVP release. The output arm of AVP involves two main peripheral receptors: V$_1r$, receptors are present on vascular smooth muscle and elicit vasoconstriction and V$_2r$ receptors are responsible for the antidiuretic properties of this hormone. They are located in the renal collecting duct and activate aquaporin-2 channels, resulting in water reabsorption. In cases of severe disease, prolific AVP release and free water resorption dilutes serum sodium concentration, and this dilutional hyponatraemia is a poor prognostic sign in both dogs (Brady and others 2004) and human beings (Gheorghiade and others 2007). AVP concentrations generally increase in human beings as cardiac disease progresses (Francis and others 1990). In human beings, the role of AVP antagonism in long-term management of heart failure is questionable. V$_1r$ or combined V$_1r$ and V$_2r$ receptor antagonists increase free water excretion and increase serum sodium concentrations, but do not delay the progression of heart failure or reduce mortality (Farmakis and others 2008, Schweiger and Zdanowicz 2008).

**ENDOTHELIN-1**

ET-1 is produced by vascular endothelial cells in response to shear stress, hypoxia, ATII and AVP. ET-1 acts primarily at ET-A receptors on vascular smooth muscle, especially within the aorta, kidneys and heart, where it increases intracellular calcium and elicits profound and sustained vasoconstriction. ET-A receptors are also found on myocardial cells where activation increases contractility. ET-1 can also bind to ET-B receptors that are located on vascular endothelium, and through formation of nitric oxide relaxes adjacent smooth muscle cells. ET-B receptors that are located directly on the vascular smooth muscle, however, elicit vasoconstriction when stimulated. Thus, ET-1 contributes to overall vascular tone through a complex arrangement of receptor types and locations. In human beings with heart disease, ET-1 levels are elevated and predictive of mortality (Pousset and others 1997, Van Beneden and others 2004). ET-1 is elevated in dogs with experimental heart failure (Ray and others 2008) as well as in dogs with DMVD or dilated cardiomyopathy (Prosek and others 2004, Tessier-Vezel and others 2006). Dogs with mild disease have ET-1 levels similar to control, suggesting that the time course of ET-1 activation is relatively late in disease (Prosek and others 2004). In human beings, ET-1 is thought to contribute to a wide array of disease conditions including pulmonary hypertension, renal disease, insulin resistance, cancer and atherosclerosis (Barton and Yanagisawa 2008). Therapy that targets ET-1 has been disappointing. ET-A receptor blockade improves haemodynamics but does not reduce mortality in human beings (Tang and Francis 2005). A recent study involving a mixed ETA and ET-B blocker was associated with early worsening followed by a trend towards improved symptoms at 6 months, but the study was prematurely discontinued due to suspected hepatic side effects (Anand and Florea 2008).

The studies involving blockade of AVP and ET-1 present an interesting conundrum. Blockade of some neurohormonal pathways improves outcome while blockade of others fails to demonstrate improvement, and may actually be deleterious. Thus far, every agent that has proven beneficial in human beings (that is, beta-blockers, ACE inhibitors and aldosterone antagonists) elicit reverse cardiac remodelling; that is, they are associated with a reduction in heart size, a return to a more normal ventricular geometry and reduced cardiac hypertrophy and fibrosis (Anand and Florea 2008; Tang and Francis 2005). In veterinary medicine, where studies seeking to prove a drug’s survival benefit are often confounded by euthanasia, small patient populations and concurrent medications, using indices of reverse remodelling as surrogate endpoints may be justifiable.

**OTHER NEUROHORMONES**

In addition to those already discussed, many other neurohormonal systems exist, and some are likely to emerge as important to the development and progression of heart disease as well as potential therapeutic targets. Cardiotrophin-1 (CT-1) is a member of the interleukin-6 superfamily and promotes myocardial hypertrophy. CT-1 is induced by myocardial stretch and its release has been shown to precede that of BNP, making it a potential marker for heart disease in human beings (Jougasaki and others 2003). Adrenomedullin is a member of the calcitonin gene-related peptide family and is found in the heart, adrenal gland and vasculature. The effects of adrenomedullin are mainly protective, that is antipapoptotic, vasodilatory, antifibrotic and diuretic (Yanagawa and Nagaya 2007). In human beings, adrenomedullin is increased in heart failure and predicts future cardiovascular events such as stroke and heart failure (Nishida and others 2008). In dogs with experimental heart failure, expression of adrenomedullin is also upregulated (Jougasaki and others 2001). Apelin is an endogenous positive inotrope and vasodilator produced by vascular endothelium and is speculated to counteract the activities of ATII (Chandrakaran and others 2008). It is downregulated in heart failure and thought to contribute to loss of contractility (Japp and Newby 2008). Urotensin II is the most potent vasoconstrictor identified to date with potency 10 times that of ET-1. Interestingly, its effects on cardiac function can include both positive and negative inotropes and either vasoconstriction or vasodilation depending on the state of the vascular bed (Russell 2008). Urotensin II is elevated in human beings with heart failure (Richards and others 2002). Urocorin is a member of the corticotrophin releasing hormone family and has been shown to protect against ischaemia and reperfusion injury (Davidson and others 2009) as well as improve heart function in animal models of heart failure (Bale and others 2004).

**CONCLUSIONS**

Activation of neurohormonal systems occurs in dogs with DMVD. Early changes consist of increased tissue activity of the SNS and possibly the RAAS, followed
by production of protective natriuretic peptides in an attempt to counterbalance the SNS and RAAS as heart disease progresses to heart failure. Along with ET-1 and AVP, the SNS and RAAS overwhelm the natriuretic peptide system and signs of congestive failure develop. By reducing activity of the SNS and RAAS overwhelm the natriuretic peptide system and signs of congestive heart failure. The Journal of Small Animal Practice 43, 26-32.


Neurohormonal activation in DMVD

cardiovascular diseases. Congestive Heart Failure (Greenwich, Conn.) 10, 1-30


