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Review

Catecholamines, cardiac natriuretic peptides and chromogranin A: evolution and physiopathology of a 'whip-brake' system of the endocrine heart

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Summary

In the past 50 years, extensive evidence has shown the ability of vertebrate cardiac non-neuronal cells to synthesize and release catecholamines (CA). This formed the mindset behind the search for the intrinsic endocrine heart properties, culminating in 1981 with the discovery of the natriuretic peptides (NP). CA and NP, co-existing in the endocrine secretion granules and acting as major cardiovascular regulators in health and disease, have become of great biomedical relevance for their potent diagnostic and therapeutic use. The concept of the endocrine heart was later enriched by the identification of a growing number of cardiac hormonal substances involved in organ modulation under normal and stress-induced conditions. Recently, chromogranin A (CgA), a major constituent of the secretory granules, and its derived cardio-suppressive and antiadrenergic peptides, vasostatin-1 and catestatin, were shown as new players in this framework, functioning as cardiac counter-regulators in 'zero steady-state error' homeostasis, particularly under intense excitatory stimuli, e.g. CA-induced myocardial stress. Here, we present evidence for the hypothesis that is gaining support, particularly among human cardiologists. The actions of CA, NP and CgA, we argue, may be viewed as a hallmark of the cardiac capacity to organize 'whip-brake' connection-integration processes in spatio-temporal networks. The involvement of the nitric oxide synthase (NOS)/nitric oxide (NO) system in this configuration is discussed. The use of fish and amphibian paradigms will illustrate the ways that incipient endocrine-humoral agents have evolved as components of cardiac molecular loops and important intermediates during evolutionary transitions, or in a distinct phylogenetic lineage, or under stress challenges. This may help to grasp the old evolutionary roots of these intracardiac endocrine/paracrine networks and how they have evolved from relatively less complicated designs. The latter can also be used as an intellectual tool to disentangle the experimental complexity of the mammalian and human endocrine hearts, suggesting future investigational avenues.

Key words: neuromodulator, catecholamine, natriuretic peptide, chromogranin A, nitric oxide, stress, poikilotherm, vertebrate heart, mammalian myocardium.

Introduction

In this article, we shall highlight the endocrine properties of the vertebrate heart in relation to catecholamines (CA), natriuretic peptides (NP) and chromogranin A (CgA). We shall sketch out some paradigms in fish and amphibians to show how studies on these animals have contributed to important evolutionary insights and have also led, and will lead, to basic and medically oriented advances. Therefore, rather than presenting the latest data, we will discuss experimental results, whether recent or not, in an integrated perspective, at the border between cardiac physiology, physiopathology and evolution, hopefully allowing the reader to use the heart as a case study for understanding the emergence of complex functions and information patterns. In updating the present knowledge, we will also refer to some last-century seminal studies performed on cold-blooded vertebrates that contributed to this concept.

From cardiac neurosecretion to the 'specific granules'

An enlightening milestone has been the development of our knowledge concerning the neural control of the heart, which led to the concept of neurosecretion. About 100 years ago, Otto Loewi arranged two frog hearts in series so that the perfusion fluid from one flowed into the second. In this way, he demonstrated that the vagal stimulation of the first heart released a vagomimetic chemical ('vagustoff'), which elicited slowing of both hearts (Loewi, 1921). At the same time, both hearts were accelerated by stimulation of the vagus 'accelerans' nerve due to release of the 'accellerans-stoff', or sympathin, later identified as adrenaline (Loewi, 1936). The discovery that the mechanical effects of electrical nerve stimulation were mediated by acetylcholine and adrenaline paved the way to our understanding of how the parasympathetic and sympathetic systems, respectively, were able to influence effector tissues. An outcome of this research, for which Loewi was awarded the Nobel Prize, was an important insight on the brain–heart connection, which integrated Walter Cannon's studies carried out in the 1930s on the role played by the autonomic nervous system and its sympathetic pathway (SNS) in the 'fight or flight' response. A few years later, Hans Selye developed the theory of the 'stress response' (or 'general adaptation syndrome') (Selye, 1936), with the consequent visceral organ dysfunction. In his further research, Selye illustrated the major role of the peripheral limbs of the stress system, the SNS and the hypothalamus–pituitary–adrenal (HPA) axis, in maintaining the stress-related homeostasis through the synergistic action of increased levels of CA and glucocorticoids

(Selye, 1956). As a paradigm of a stressed-injured organ, he described the electrolyte-steroid cardiomyopathy, which revealed the heart not only as a complex self-regulating mechano-chemical transducer but also as an integrative interface between the autonomic nerve terminal-released neurotransmitters and the circulating endocrine-humoral substances.

Within this framework, the pioneering studies of the late 1950s promoted the beginning of the endocrine story (*sensu stricto*) of the vertebrate heart. First, in the heart of *Lampetra fluvialis*, Augustinsson et al. (Augustinsson et al., 1956) re-described the granule-containing cells, i.e. the chromaffin cells, reported by Gaskell as early as 1912 (Gaskell, 1912). Johnels and Palmgren (Johnels and Palmgren, 1960) demonstrated their presence in the cyclostome (*Myxine glutinosa*) heart, which was shown to contain large amounts of CA (especially adrenaline) (Ostlund et al., 1960; von Euler and Fänge, 1961). At the same time, within the mammalian atrial myocardium, many studies documented the presence of dense core granules resembling, both morphologically and functionally, those of the endocrine cells (Kisch, 1956; Bompiani et al., 1959; Palade, 1961; Jamieson and Palade, 1964; de Bold and Bencosme, 1973; de Bold, 1979). Soon after these first reports, a number of comparative studies highlighted the evolutionary roots of these granules in cold-blooded vertebrates, demonstrating their presence in atrial and ventricular myocardial cells as well as in the endocardial cells lining the cardiac lumen (Santer and Cobb, 1972; Helle et al., 1972; Helle and Lönning, 1973; Cantin et al., 1979; Helle et al., 1983). An important outcome of these studies was the demonstration of elements of the diffuse neuroendocrine system in the heart of CA-producing cells, mostly corresponding to chromaffin cells. These were located singly and/or in clusters in different atrial and ventricular regions, particularly in the subendocardium, suggesting either a direct release of CA into the intracavitary luminal blood and/or a paracrine control of the subjacent myocardium. The latter encapsulated a seminal idea that, as illustrated below, became experimentally fruitful only in the 1990s, during the rise of the nitric oxide (NO) era, following Furchgott and Zawadski's discovery of endothelium-derived relaxing factor (EDRF) (Furchgott and Zawadski, 1980), i.e. the obligatory role of vascular endothelium in the vasomotor tone of the subjacent smooth muscle.

Cardiac hormone discovery

The postulated endocrine function of the 'specific granules' was supported by Marie et al., who showed, in the myoendocrine cells of the rat heart, the relationship between granular content and the water and salt balance (Marie et al., 1976). However, it was only in 1981 that de Bold and co-workers revealed the endocrine function of the constituent of these granules by preparing, from 100 rat atria, an extract that, infused in rats, caused diuresis and natriuresis (de Bold et al., 1981). The unknown substance was named atrial natriuretic factor (ANF), later isolated and characterized as a 28-amino-acid peptide called atrial natriuretic peptide (ANP) (Flynn et al., 1983; de Bold and Flynn, 1983). Soon after, another two natriuretic peptides (NP) – brain natriuretic peptide, now called B-type natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) – were identified (Sudoh et al., 1988; Sudoh et al., 1990). Other identified and sequenced NP were the VNP (ventricular natriuretic peptide), found in the eel ventricle (Takei et al., 1991), and DNP (*Dendroaspis* Natriuretic Peptide), found in the venom of the green mamba snake (*Dendroaspis angusticeps*) (Schweitz et al., 1992). Present in the hearts of a large number of non-mammalian vertebrates (Netchitailo et al., 1987; Takei et al., 1990; Bjenning et al., 1992; Larsen et al., 1994; Kawakoshi et al., 2003), as well as in invertebrates and plants, NP regulate ion fluid and circulatory homeostasis, which in vertebrates includes major cardiac and vascular actions.

Clearly, the term 'natriuretic peptide' has become synonymous with cardiac hormones. However, as reported in Table 1, in the past two decades the identification of a large number of other substances produced by cardiac cells has broadened the scenario of the endocrine heart. Although in many cases their endocrine and/or paracrine/autocrine role still waits a conclusive demonstration, this extremely differentiated capacity of the heart offers new insight into understanding cardiac autoregulation and neurovisceral integration. Functioning in concert with the neuro-transmitters released by the intracardiac autonomic nervous terminals, these endocrine substances increase dramatically the variety of chemical signals to be recruited and integrated in complex networks that finely tune cardiac performance to local and/or systemic challenges. To hallmark the endocrine properties of the vertebrate heart, we will focus only on three substances, i.e. the CA, the NP and the CgA-derived peptides vasostatin and catestatin. Being co-stored in granule-containing cells and often released in response to specific excitation–secretion stimuli, these three endocrine substances and their actions point to a striking morpho-functional framework for an excitatory/counter-regulatory loop system that may dynamically stabilize the heart in response to often dramatic internal and environmental challenges. An impressive proliferation of relevant research on the molecular biology of cardiac hormones, their receptors and regulation, often biomedically oriented, has been performed on mammals, thus providing an advanced and fundamental theoretical point of reference. Consequently, when necessary, we will refer to it, so that evidence accumulated regarding poikilotherm vertebrate hearts can be better perceived.

The basic fish and amphibian hearts

For anyone not familiar with the functional morphology of the fish and amphibian heart and the pertinent anatomical terminology, we will briefly summarize some basic traits related to the issues of this review. As illustrated by zebrafish (*Danio rerio*), a major model for vertebrate embryology and genomics, the fish heart is the prototype of the higher vertebrate hearts (Fishman and Stainier, 1994). It consists of four chambers in series: the sinus venosus, the atrium, the ventricle and the outflow tract (bulbus cordis). In a typical water-breathing fish, the peripheral venous blood flows in sequence from the sinus venosus to the atrium, to the ventricle and to the bulbus cordis, from where the venous blood is pumped to the gills to be oxygenated and then distributed to the body, reflowing to the heart.

In most teleosts and in the sarcopterygian lungfish, the ventricle is made up of projecting cones of myocardial muscle, the 'trabeculae', and is named 'spongiosa' because of its spongy texture. It is supplied by the intracavitary venous blood, which perfuses the inter-trabecular (lacunae) spaces. In elasmobranchs and many teleosts, the spongiosa is covered by a subepicardial outer layer of densely arranged myocardial bundles, the 'compacta', thus forming a 'mixed type' of ventricle (Tota, 1983; Tota et al., 1983; Icardo et al., 2005). In a variety of species, the entirely trabeculated ventricle, in addition to the lacunary supply, can receive a vascular supply; the compacta is always vascularized (Tota et al., 1983; Tota, 1989; Farmer, 1997). In comparison with the homeotherm heart, the fish heart generally functions as a low-pressure region, facing relatively low and variable oxygen partial pressure (P_{O_2}) levels (Farrell and Jones, 1992; Olson, 1998). Among the

Table 1. Substances produced by the vertebrate heart

Substance	Site of production	Reference
Adenylpurines	Endothelial cells and cardiac myocytes	Shah, 1996
Adrenomedullin	Cardiac myocytes and fibroblasts (atria and ventricles)	Kato et al., 2003
Aldosterone	Atria and ventricles (rat)	Delcayre et al., 2000
Angiotensin II	Cardiac and vascular RAS	Dzau, 1993
Apelin	Neonatal heart	Katugampola et al., 2001
Calcitonin gene-related peptide	Atria, sinoatrial and atrioventricular nodes	Beaulieu and Lambert, 1998
Catecholamines	Myoendocrine, vascular and endocardial endothelial cells, intracardiac chromaffin cells, ICA (intrinsic cardiac adrenergic cells)	Burnstock, 1969; Abrahamsson et al., 1979; Larsen et al., 1994; Huang et al., 1996; Huang et al., 2005; Saetersdal et al., 1975
Chromogranin A and derived peptides	Atrial and ventricular cardiocytes, conduction system	Steiner et al., 1999; Weiergraber et al., 2000; Pieroni et al., 2007
Coenzyme A glutathione disulfide (CoASSG)	Myocardial tissue	Luo et al., 2006
Cytokines	Cardiac myocytes and coronary endothelium	Kaye et al., 1996
Endothelin-1	Coronary endothelium and cardiac tissue	Shah, 1996; Beaulieu and Lambert, 1998
Ghrelin	Cardiomyocytes	Iglesias et al., 2004
Leptin	Cardiomyocytes	Purdham et al., 2004
Neuropeptide Y	Nodal tissue, atria and coronary vessels	Beaulieu and Lambert, 1998
Nitric oxide	Endothelial cells and cardiac myocytes	Shah, 1996
Oxytocin	Cardiac tissue (rat)	Jankowski et al., 1998
Proadrenomedullin N-terminal 20 peptide (PAMP)	Cardiac myocytes and fibroblasts (atria and ventricles)	Kato et al., 2003
Prostanoids	Endothelial cells and cardiac myocytes	Shah, 1996
Relaxin	Atrial cardiomyocytes (rat)	Taylor and Clark, 1994
Substance P	Sinoatrial and atrioventricular nodes	Beaulieu and Lambert, 1998
Urocortin	Cardiomyocytes and non-cardiomyocytes	Ikeda et al., 2002
Vasoactive intestinal peptide (VIP)	Sinoatrial node and coronary vessels	Beaulieu and Lambert, 1998

vertebrates, teleost fish exhibit the highest interspecific variation not only in myo-angio-architecture but also in patterns of morphodynamic mechanical performance (Farrell and Jones, 1992; Tota and Gattuso, 1996).

Amphibians possess an entirely trabeculated and, in most groups, avascular heart, consisting of a sinus venosus, right and left atria divided by an anatomically complete internal septum, a ventricle lacking any internal subdivision and a conus arteriosus with a spiral valve. In the single ventricle, the ‘venous’ and the more ‘arterial’ blood remain to a large extent unmixed, to be distributed in the low-pressure pulmo-cutaneous arch (venous blood), the systemic arches (mixed blood) and the high-resistance carotid arches during different systolic phases (Foxon, 1964; Kardong, 1995). The remarkable variety of respiratory and circulatory patterns found in their orders – the Apoda, the Urodela and the most numerous Anura – prevents any generalization regarding the mode of action of the ‘amphibian heart’ (Foxon, 1964). However, since the major endocrine evidence concerns the Anura, only the heart of *Rana* will be considered here as a paradigm.

The endocrine function of the endocardial endothelium

A striking feature of the avascular spongy ventricle is the impressively large surface area of the endocardial endothelium (EE) that covers the lumenal cavity and the ‘lacunae’. Analogous to the vascular endothelium (Furchgott and Zawadski, 1980) in mammals [see Brutsaert (Brutsaert, 2003) and references therein], the EE is well suited to detect physical and chemical intracavitary stimuli and to transduce them on the subjacent myocardium. Thanks to the large numbers of receptors for endocrine/growth factors and its autocrine–paracrine ability (e.g. *via* EE–NOS/NO-mediated signalling; see below), the EE acts as a sensor-integrator device, to be viewed as short- and medium-term memory of intracavitary signalling. For cardiac morphogenetic remodelling, see the article by Hove et al. (Hove et al., 2003) and references therein. Fish and amphibian hearts offer a repertoire of natural models for analyzing the EE–myocardium axis, providing unique opportunities for understanding its early ontogenetic and phylogenetic roots, as well as how it influences medium- and long-term changes of the heart.

Catecholamines

CA, typically adrenaline and noradrenaline, exert relevant actions on the metabolic, contractile and excitation–conduction properties of the vertebrate heart. According to the pertinent terminology of myocardial mechanics, the terms ‘inotropism’ and ‘lusitropism’ will be used; namely, increased or decreased inotropism is equivalent to increased or decreased force of contraction, while positive or negative lusitropism indicates enhanced or reduced relaxation. In the 1960s, Sonnenblick demonstrated that, apart from their positive action on heart rate (f_H), CA both enhance the rate of force development and develop contractility at any given muscle length (Sonnenblick, 1962). CA also accelerate the rate of relaxation, decrease the duration of contraction and increase myocardial oxygen consumption (e.g. the so-called ‘oxygen wasting’ effects of noradrenaline) (Sonnenblick, 1962). These short-term ‘whip’ actions, paralleled by those exerted on the vasculature, are crucial for pre-adjusting the cardiovascular system to match the systemic demands required by the ‘fight or flight’ response. Concomitantly, the stress response also triggers a sustained heightened activation of the renin–angiotensin system (RAS) and the angiotensin II (ANG II)–endothelin-1 (ET-1) signaling. However, if left uncontrolled, these powerful excitatory cascades may lead to critical homeostatic transitions at which a

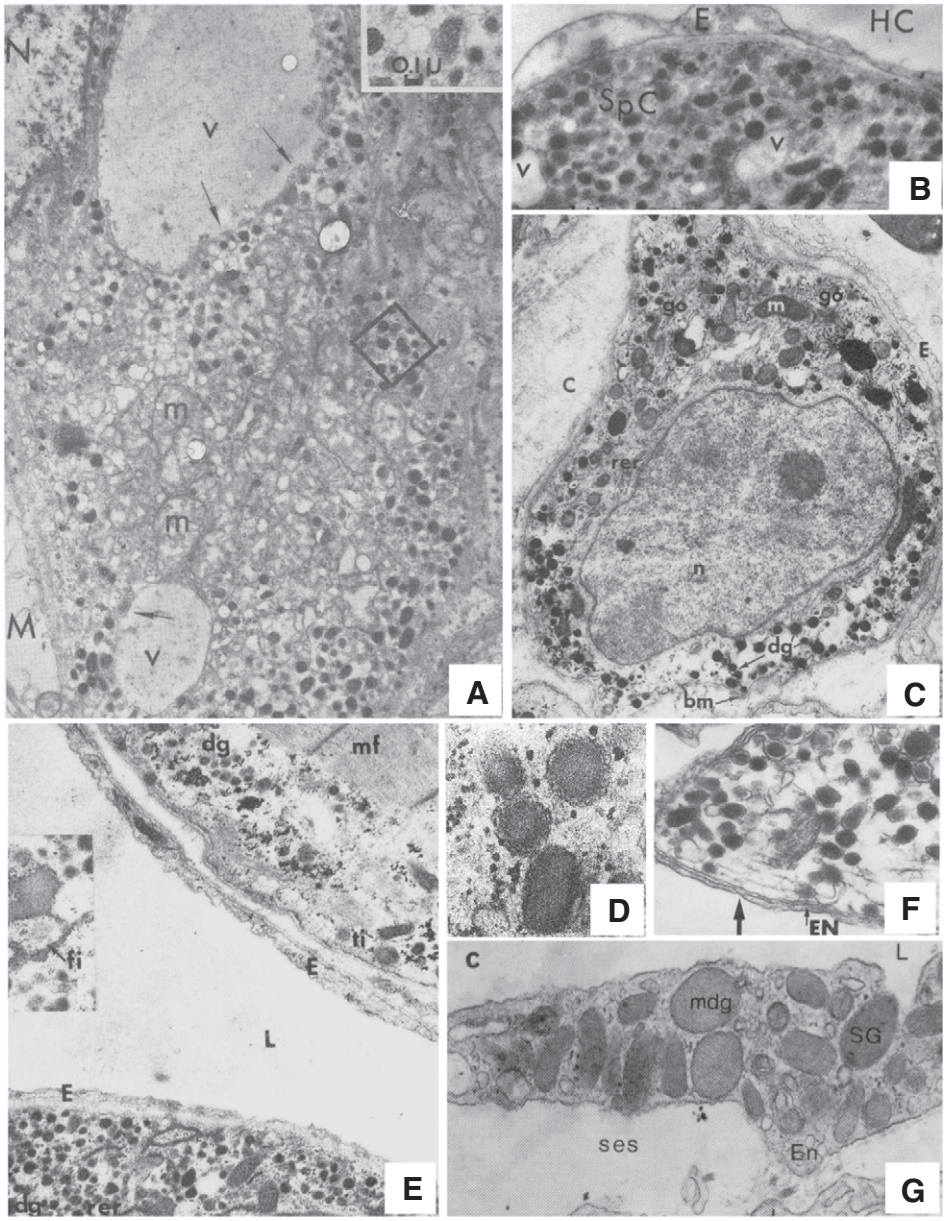


Fig. 1. Location of electron-dense granules in several vertebrates. (A,B) *Petromyzon fluviatilis* (ventricular cell, A; atrium, B); (C–E) *Myxine glutinosa* (portal vein heart, C,D; ventricle, E); (F) *Galeus melastomus* (sinus venosus); (G) *Scyllium stellare* (ventricular endocardium). The inset in A shows a magnification of the granules. Abbreviations: bm, basement membrane; C, collagen; dg, dense granules; E, endothelial cell (in B), endothelium (in C,E); En, endocardium; EN, endothelium; fi, filamentous inclusions; go, Golgi apparatus; HC, heart cavity; L, lumen; m, mitochondria; M, muscle cell; mdg, moderately dense electron granules; mf, myofibrils; n, nucleus; N, nucleus; rer, rough endoplasmic reticulum; SG, specific granules; SpC, specific granules; ses, subendocardial space; ti, tubular invaginations; v, vacuoles. For magnification, see original articles [modified from (A,B) Bloom et al. (Bloom et al., 1961), (C–E) Helle et al. (Helle et al., 1972), (F) Saetersdal et al. (Saetersdal et al., 1975) and (G) Helle et al. (Helle et al., 1983)].

contrasting process may occur. Namely, in the deterioration process, such overstimulation may be more important than the actual stress placed on the heart and the vasculature (Samuels, 2007). As part of this scenario, these stimulatory cascades contribute to important long-term readjustments that affect heart physiopathology (hypertrophic growth, ischemia and myocardial failure) (Samuels, 2007). Indeed, such concepts provided the rationale in humans for anti-adrenergic drug therapy, including the still most used β-adrenergic-blockers.

Adrenergic neuroendocrine patterns

CA reach cardiac α- and β-adrenoceptors (ARs) *via* both the circulation and the SNS terminals, when present. From fish to reptiles, the heart increases and diversifies its innervation. Moving from the aneural heart of the hagfish, or the presence of only cholinergic nerves in lampreys and elasmobranchs, it reaches an establishment of both sympathetic and parasympathetic nerves in teleosts and amphibians. In comparison with those of birds and mammals, the SNS of these vertebrates is less developed because of the lack of longitudinally connected sympathetic chains (Laurent et al., 1983; Taylor, 1992). The peripheral nerves are replaced by aggregates of CA-containing chromaffin cells, which in many species become components of the diffuse neuroendocrine tissue (Burnstock, 1969). The strategic location of these aggregates in hemodynamically important cardiovascular regions, such as the wall of the large systemic veins (cyclostomes, teleosts, lungfish), the axillary bodies (elasmobranchs) or embedded in the cardiac muscle itself (teleosts, elasmobranch, lungfish, amphibia), highlights a major paracrine sensory (detector)–secretory (effector) axis (Fig. 1). Being often associated with sympathetic nerves and/or receiving cholinergic stimulation, these chromaffin cells provide a zonal CA production, thereby contributing to the humoral cardiovascular regulation (Gannog and Burnstock, 1969; Abrahamsson et al., 1979; Tota, 1999). An example is represented by elasmobranchs and Dipnoi, which, for their lack of direct cardiac sympathetic control, depend on the CA-induced modulation of myocardial inotropism and f_H (chronotropism) achieved by the activation of cardiac chromaffin cells. The identification of these

intracardiac sites for CA production and release in poikilotherms offered intriguing morpho-functional and biochemical hints to the precursor of neuroendocrine and humoral cardiac networks, enlightening how the concept of the endocrine heart has evolved.

CA-induced autocrine/paracrine effects: a need for a re-evaluation

The diverse sources and topological localization of cardiac CA are often mirrored by the distinct responses they elicit, ranging from the insusceptibility of the myxinoid heart to the complex, and often non univocal, sensitivity shown by teleosts and amphibians. Therefore, while a general evolutionary trend can reasonably be traced, the importance of inter- and intraspecific differences must be considered. Moreover, by retrospectively reinterpreting the past studies on the basis of the present knowledge, their univocal explanation may be hampered by some theoretical and methodological aspects. One is the impact of stress and stress responses in lower vertebrate physiology and adaptation, now widely recognized (Cossins et al., 2006; Johnsson et al., 2006). Accordingly, caution is required in accepting conclusions of many studies performed without rigorous quantitative evaluation of the stress-induced changes imposed by the experimental conditions upon either the organism or the heart preparation. This is indeed crucial when the major stress effector molecules, i.e. the CA, are under study. As discussed by Epple and Brinn, another limitation has been the use of pharmacological but not physiological concentrations of CA (Epple and Brinn, 1987). This precluded the identification of the today-acknowledged yin–yang modulation (e.g. the biphasic inotropic response) exerted by CA as components of the complex adrenoceptor–G-protein-coupled signal-transduction pathways, including NO–cGMP and cAMP axes (see below). On the basis of the most advanced mammalian knowledge, these pathways represent major regulators of almost every aspect of cardiac performance, a concept that too slowly is going to be incorporated in fish cardiology [for references, see Tota et al. (Tota et al., 2007b)].

CA in fish hearts: evolutionary and heurystic hints

Fish hearts and the hormones they secrete are particularly interesting because various species show different degrees of cardiac myoarchitecture, blood supply and innervation, thus appearing as critical intermediates during evolutionary transitions or in a distinct phylogenetic lineage. Some of these paradigms will be illustrated to show how the CA-related humoral networks evolved from relatively less complicated designs that cannot be found in the adult homeotherm heart.

The lampetroid heart highlights an early evolutionary response to CA (Nilsson, 1983). The pioneering study of Ostlund et al. (Ostlund et al., 1960) suggested an intracardiac CA production, which was later identified in atrial and ventricular CA-containing fluorescent granules (Otsuka et al., 1977). This cardiac chromaffin tissue constitutes an intrinsic control system that releases adrenaline in response to intracavitary stimuli. In turn, adrenaline stimulates noradrenaline, and probably also dopamine release from other cardiovascular chromaffin cells (Dashow and Epple, 1985). Noradrenaline targets the myocardium *via* β-Ars, providing CA stimulatory actions that, however, seem less powerful than those elicited by acetylcholine (ACh) [for references, see Taylor (Taylor, 1992)].

In elasmobranchs, Saetersdal et al. demonstrated that the heart is influenced, either directly or indirectly, by a basal CA release from intracardiac stores, as well as from suprarenal and axillary bodies (Saetersdal et al., 1975). The predominant trigger for CA secretion is ACh released by preganglionic fibers of the SNS [see Nilsson et al. and others (Nilsson et al., 1976; Randall and Perry, 1992) and references therein]. Physical disturbance, exercise and hypoxia represent major stimuli for increasing plasma CA levels in dogfish (Opdyke et al., 1982; Metcalfe and Butler, 1989; Taylor, 1992). The direct modulation is exerted either by CA released by the axillary bodies and immediately sucked into the heart during each cycle or by those released by the stores localized in the sinus venosus, in the atrium and in the ventricle. The resulting positive chronotropic and inotropic effects (Capra and Satchell, 1977) are achieved *via* β-ARs, resembling mammalian β2-ARs (Ask, 1983). Plasma CA indirectly regulate cardiac filling pressure *via* an α-ARs-mediated increase of the venous pressure (*Squalus acanthias*) (Sandblom et al., 2006) and by a presynaptic modulation of the vagal inhibition (*S. acanthias*) (Agnisola et al., 2003). Moreover, CA modify the vasoactivity of the coronary system [well-developed in elasmobranchs (Tota et al., 1983)], thus adjusting coronary performance to myocardium requirements (Axelsson, 1995). An electrophysiological study in sharks (Woo and Morad, 2001) showed that, in the presence of very limited intracellular Ca^{2+} stores, likely due to a poorly developed sarcoplasmic reticulum, the myocardial contraction elicited by β-ARs stimulation requires a time-dependent modulation of the Ca^{2+} transients through the Na^{+}–Ca^{2+} exchanger. This initially contributes to increased Ca^{2+} entry and subsequently facilitates Ca^{2+} efflux, thus accelerating CA-dependent relaxation (Woo and Morad, 2001).

The air-breathing lungfish (Dipnoi) represent ideal models to study the evolution of cardiac adrenergic control in vertebrates. Their autonomic nervous system is less differentiated than in teleosts and appears gradually adapted for terrestrial life. This is suggested by morpho-functional observations related to the endocrine–paracrine requisites of their cardiovascular CA-mediated control. In several lungfish species, e.g. the African *Protopterus aethiopicus* and *Protopterus annectens*, as well as the genus *Lepidosiren*, chromaffin cells produce a primary CA identified as dopamine, being located singly and/or in a cluster in the wall of the sinus venosus and in the auricle [for references, see Abrahamsson et al. (Abrahamsson et al., 1979) and Larsen et al. (Larsen et al., 1994)]. Identical to those found in several autonomic ganglia of a variety of vertebrates and situated in the position where chromaffin cells or their precursors locate in mammals during ontogenetic development (Scheuerman, 1993), these cells represent an intermediate phenotype between mature chromaffin cells and primitive sympathetic cells. Of note, CA-producing cells populate subendocardial areas of the atrium, suggesting either a direct release of CA into the intraluminal blood or a paracrine control of the subjacent myocardium (Larsen et al., 1994; Fritsche et al., 1993). So far, few studies have analysed the responses of the lungfish heart to CA. However, it may be expected that in these animals the CA-dependent stimuli are of relevance. In fact, during the dry tropical season, they tolerate drought periods by aestivating in subterranean mud cocoons (Smith, 1935; Janssens and Cohen, 1968). This requires cardio-respiratory and metabolic changes, including complete reliance on air-breathing with a consequent reorganization of the branchial/lung vascular perfusion, decreased oxygen consumption, slowing of f_H and a drop in blood pressure [for references, see Amelio et al. (Amelio et al., 2008)]. In *Protopterus dolloi*, during terrestrial aestivation, plasma CA level does not change (Perry et al., 2008), while during exposure to aerial hypoxia the fish is able to mobilize stored CA (Perry et al., 2005). During aerial hypoxia, the increased plasma CA level is not accompanied by f_H variations (Perry et al., 2005). Whether, and to

what extent, these mobilized CA exert short- or medium-term cardiac or vascular protection is unknown. However, since cardiac ultrastructural changes occur during aestivation (Icardo et al., 2008), it should be explored whether either circulating or locally produced CA exert long-term actions on the lungfish heart.

Teleosts comprise a number of species that far exceeds those of any kind of vertebrate, representing 96% of living fishes and more than half of all vertebrates (Bone et al., 1995). They were the first vertebrates to receive cardiac sympathetic innervation *via* 'vago-sympathetic' trunks (Laurent et al., 1983; Nilsson, 1983; Taylor, 1992). Such a double nervous control increases the adaptive and acclimatory potentialities of the heart of many teleosts to face changes in environmental salinity, extreme temperatures, variable oxygen availability, sustained enforced activity, etc.

Based on the limited number of species studied, it is generally believed that in teleosts, under resting conditions, plasma CA levels are low, the nervous activity playing a major tonic role on the heart (Axelsson, 1988). CA exert a basal excitatory tone that prevails over the cholinergic tone. Adrenergic tone is mediated by α- and β-ARs associated with both the pacemaker and the working myocardium (Axelsson et al., 1987; Gamperl et al., 1994). Adrenergic stimulation increases f_H (Graham and Farrell, 1989) and slightly improves the Frank–Starling response (Farrell et al., 1986). Recent evidence has made this picture more complex, identifying in two teleost species a novel type of cardiac β-ARs (β3). In the trout *Oncorhynchus mykiss*, β3-ARs are richly expressed in the heart and are homologous to their mammalian counterparts (Nickerson et al., 2003). In mammals, β3 activation elicits negative inotropism [for references, see Gauthier et al. (Gauthier et al., 2000)] and negative lusitropism (Angelone et al., 2008a), both involving NO-cGMP signaling. Similarly, in *Anguilla anguilla*, β3-ARs activation decreases cardiac mechanical performance through a pertussis toxin (PTx)-sensitive G_i protein mechanism, consistent with a major β3-ARs myocardial localization, and requires the NO-cGMP-cGMP-activated protein kinase (PKG) cascade (Imbrogno et al., 2006). Therefore, CA may regulate fish cardiac performance in a yin–yang fashion that is much more complex than hitherto perceived. Whether such an intrinsic β3-ARs inhibitor tone exerts cardio-protection against the stress-induced excessive excitatory stimulations (exposure to systemic and/or intracardiac CA, angiotensin, endothelin) remains a closed book.

The amphibian heart: a case study from sympathin on....

As in many fields of developmental biology and physiology, amphibians represent powerful natural tools for investigating neuroendocrine mechanisms such as those converting environmental signals into physiological responses. The frog heart has paved the way for basic advances in cardiac physiology, as epitomized by the 'Frank–Starling law of the heart' and Otto Loewi's studies mentioned above.

CA hormones, being synthesized and stored in the non-innervated heart during its early developmental stages, may function as cardiac paracrine modulators. This is exemplified by the heart of *Xenopus laevis* larvae, in which the content of adrenaline, noradrenaline and dopamine increases during growth (Kloberg and Fritsche, 2002), paralleled by a growth-dependent increased adrenergic tone responsible for the high f_H occurring during late development (Jacobsson and Fritsche, 1999). In the adult, this CA-dependent cardiostimulation is maintained (Jacobsson and Fritsche, 1999). In *Bufo marinus*, both intracellular calcium concentration and firing rate are increased by β-ARs stimulation of pacemaker cells (Ju and Allen, 1999). f_H is stimulated by non-neural CA (including those released by intracardiac stores) through activation of extrajunctional β-ARs linked to a cAMP-dependent metabotropic pathway. By contrast, CA released by sympathetic fibers seem to activate a set of dihydroergotamine-sensitive non-α-, non-β-adrenoceptors that are linked to Ins(1,4,5)P_3-dependent signaling, analogous to the chronotropism controlling mechanism in mammals (Bramich et al., 2001).

Routinely, isoproterenol (ISO) is used to study the effects of β-ARs stimulation on myocardial contractility, although adrenaline is considered as the typical amphibian cardiac CA. The frog myocardium shows some of the features associated with the ISO response, including potentiation of phasic contraction (twitch) followed by an inhibition of tonic (maintained) tension [see Fan et al. (Fan et al., 1996) and references therein], which is known as the 'paradoxical' cardiac action of adrenaline (Graham and Lamb, 1968). Moreover, as shown on isolated ventricular strips of the toad, ISO increases the rate of relaxation. The β2-ARs-dependent decreased myofibrillar sensitivity to Ca^{2+} accounts for both inotropism and lusitropism (Petroff et al., 1994; Fan et al., 1996). Importantly, frog ventricular myocytes have provided a key paradigm for dissecting the mechanisms of CA-induced modulation of contractility, detailing the functional coupling between β2-ARs and Ca^{2+}. In amphibians, as in many elasmobranchs and teleosts, myocardiocytes show poor sarcoplasmic reticulum (SR) and limited Ca^{2+}-ATPase/phospholamban (PLN) complex. They also lack significant intracellular Ca^{2+} pools. Thanks to a highly compartmentalized pool of phosphodiesterases (PDEs) (i.e. PDE3 and PDE4 equally active in the membrane fraction, and PDE4 mainly active in the cytosol), Jurevicius et al. demonstrated that β2-ARs activation modifies the intracellular spatial profile of camp and the activity of cAMP-dependent protein kinase (PKA), and thus of its closely localized substrates, including L-type Ca^{2+} channels (Jurevicius et al., 2003). This, in turn, determines the amount of L-type calcium currents, influencing contractility. Moreover, an ISO-dependent downregulation of the transmembrane Ca^{2+} influx through the 'reverse mode'-operating Na^+/Ca^{2+} exchanger (Campbell et al., 1988) was suggested to contribute to the β-ARs inhibition of the tonic tension and the positive lusitropism (Fan et al., 1996). The identification of these fine-tuned mechanisms provided an important insight into the ways by which the myocardial cells respond to various external stimuli, modulating the amount of a single intracellular messenger according to a restricted spatio-temporal pattern.

CA, stress and CA-induced cardiotoxicity

From the considerable number of studies performed in teleosts, it appears that under stress challenges, such as hypoxia, air exposure, anemia, acidosis, hypercapnia, exhaustive exercise and physical disturbances, there is a sudden release of CA from chromaffin cells, such as those richly embedded into the walls of the posterior cardinal vein close to the head kidney (Nandi, 1961). Accordingly, the heart may become targeted at the same time by these blood-borne CA and those released by both SNS terminals and cardiac chromaffin cells (Nilsson and Holmgren, 1992; Farrell and Jones, 1992). In addition to their cardioexcitatory effects, CA activate vascular and respiratory responses, thus helping to alleviate stress-dependent detriments (Farrell et al., 1986). Laboratory studies in simple environments suggest that, similar to those described in rodents and even in humans, divergent stress-coping strategies exist in rainbow trout [see Schjolden et al. (Schjolden et al., 2005) and references therein], i.e. proactive and reactive styles (van Raaij et

al., 1996). Under hypoxia, the proactive style, corresponding to a non-surviving fish, is characterized by strenuous avoidance behavior, while the reactive style remains calm and survives. Strikingly, plasma CA levels are 4–5-fold higher in the former compared with the reactive calm counterpart. These differences are very similar to the active and passive coping strategies observed in other vertebrates including mammals (van Raaij et al., 1996). Considering that another excitatory system, the RAS, is also stress-activated and synergizes with CA, we may ask whether the teleost heart is able to protect itself from these potentially exaggerated excitatory cascades. This fundamental evolutionary question will be considered below in relation to NP and CgA-derived peptides.

In both poikilotherms and homeotherms, excessive CA provoke serious structural and functional myocardial lesions, in the so-called 'sympathetic storm' (Samuels, 2007). The ventricular non-uniformity of many poikilotherms has, for years, provided appropriate experimental conditions to discriminate between vascular (coronary)- and myocardial-dependent reactions induced by CA toxicity. For example, in the turtle *Testudo horsfieldi*, 48 h ISO administration caused necrosis only in the avascular spongy ventricular myocardium, while the outer compact and vascularized layer remained intact (Ostádal et al., 1968). This zonal vulnerability, which resembles the subendocardial vulnerability detected in mammalian (and human) hearts (Samuels, 2007), appears of particular interest since the reptilian myocardium is usually anoxia-resistant (Gesser and Poupa, 1978). Fishes (i.e. trout and tuna) showed a lower susceptibility to ISO-induced lesions (Poupa and Ostádal, 1969; Ostádal and Rychterova, 1971). The amphibian myocardium seems even less susceptible. However, the resistance to CA toxicity is strongly broken down by increasing environmental temperature, thus showing a seasonal variation (Poupa and Carlsten, 1970). In frogs, CA-elicited myocardial lesions mainly localize in the ventricle at the level of the base and the apex and often associate with paradoxical systolic movements of the aneurysmatic ventricular wall. The damaged ventricle shows altered force development and resistance to anoxia, together with degeneration of the trabecular arrangement, edematous myocytes with enlarged mitochondria, disarranged myofibrils and contraction bands [for references, see Carlsten et al. (Carlsten et al., 1983a) and Garafolo et al. (Garofalo et al., 2006)].

Apart from species-specific differences, the notable resistance against the cardiotoxic action of CA makes the cold-blooded vertebrate heart well suited to study tissue, cellular and molecular mechanisms of cardioprotection. To some extent, this natural cardioprotection may relate to the lower levels of temperature, f_H, contraction velocity and oxidative metabolism, for which the work of the poikilotherm heart is less intense and oxygen-dependent than that of most homeotherms. This may help against the unfavorable influence exerted by the intracardiac and/or circulating CA.

Natriuretic peptides

As for other fish hormones, sequence analysis has tracked the evolutionary roots and relationships of the natriuretic peptides (NP), so far the best-characterized cardiac endocrine products in vertebrates.

After de Bold and co-workers identified 'atrial natriuretic factor' (ANF) as a major constituent of rat atrial granules with potent diuretic and natriuretic effects (de Bold et al., 1981; Cantin and Genest, 1985), NP were soon recognized as a ubiquitous hormonal system in both mammalian and non-mammalian vertebrates. NP represent crucial components of the homeostatic loop that orchestrates ion/fluid and circulatory balance by linking blood volume expansion and myocardial stretch. The consequent cardiovascular regulation is achieved *via* concerted multi-target effects on the heart, vasculature, kidney, adrenal glands and central and autonomic nervous systems. At the same time, NP are involved in some regulatory programs that control cardiac morphogenesis and adult heart remodelling. Therefore, they increase the efficiency of information and 'memory' of the heart by interacting with other neuropeptides and endocrines produced by other cell components (Hirose et al., 1998; Zhang et al., 2005), improving the zonal capacity to detect and respond to variations in the internal and external environment. We will illustrate, from a phylogenetic and biomedical perspective, the major NP actions, also summarizing their important interactions with CA.

NP–NPR system: molecular and evolutionary strategies

The basic NP structure consists of a highly conserved 17-amino-acid intramolecular ring and extending N- and C-terminal sequences of different lengths (Takei and Hirose, 2002). The C-terminus is absent in CNP but is very long in VNP and DNP (Fig. 2). ANP and CNP are the best-conserved NP. In particular, all mammalian CNP so far sequenced are identical, except the one isolated from the venom of egg-laying platypus (de Plater et al., 1998). The most variable NP is BNP; its sequence identity is only 35% between human and mouse (see Takei and Hirose, 2002).

Universally distributed (Table 2), NP are present in single-cell organisms (Vesely and Giordano, 1992), plants (Vesely and Giordano, 1991; Yang et al., 1999; Billington et al., 1997; Pharmawati et al., 1998) and invertebrates (Brownlee et al., 1993; Kim et al., 1994), including mollusks (Reinecke et al., 1989; Poulos et al., 1995). Their ubiquitous expression, conserved functions and high sequence homology indicate a long evolutionary history. NP appeared 565 million years ago as an ancestral peptide, presumably CNP, and diverged into four groups of structurally similar molecules. About 360 million years ago, their divergence gave rise to the sequences present also in humans (Inoue et al., 2003a). This phylogenetic history is documented by the variable expression of NP among vertebrates. A single peptide is present in the hagfish and in elasmobranches: EbuNP and CNP, respectively (Kawakoshi et al., 2003; Suzuki et al., 1994; Takei, 2000). By contrast, in the sturgeon *Acipenser transmontanum*, all ANP-, BNP-, CNP- and VNP-encoding genes are present (Kawakoshi et al., 2004). With the exception of a few species – medaka, which do not possess ANP, and eel, which lack BNP (Takei et al., 1991; Takei et al., 1994a; Takei et al., 1994b) – all NP are expressed in teleosts. Recently, BNP was also detected in the rainbow trout (Johnson and Olson, 2009b). Tetrapods typically possess ANP, BNP and CNP. ANP and BNP exist in amphibians and mammals, while birds apparently lack ANP [see Takei (Takei, 2000) for references] but possess an NP highly expressed in the kidney [renal NP (RNP)] (Trajanowska et al., 2007; Trajanowska and Donald, 2008). Mammals and reptiles also express DNP (Schirger et al., 1999; Kim et al., 2004; Woodard et al., 2002).

In parallel with the peptides, a complex NP receptor (NPR) system differentiated during evolution. Currently, four molecules form this system: NPRA to NPRD. These receptors, present from cyclostomes to mammals [for references, see Takei and Hirose (Takei and Hirose, 2002), Toop and Donald (Toop and Donald, 2004) and Garg and Pandey (Garg and Pandey, 2005)], conserve molecular and functional features. NPRA and NPRB are single-spanning transmembrane receptors, belonging to the family of particulate guanylate cyclases (pGC) [for references, see Cerra and Pellegrino (Cerra and Pellegrino, 2007)]. They show different

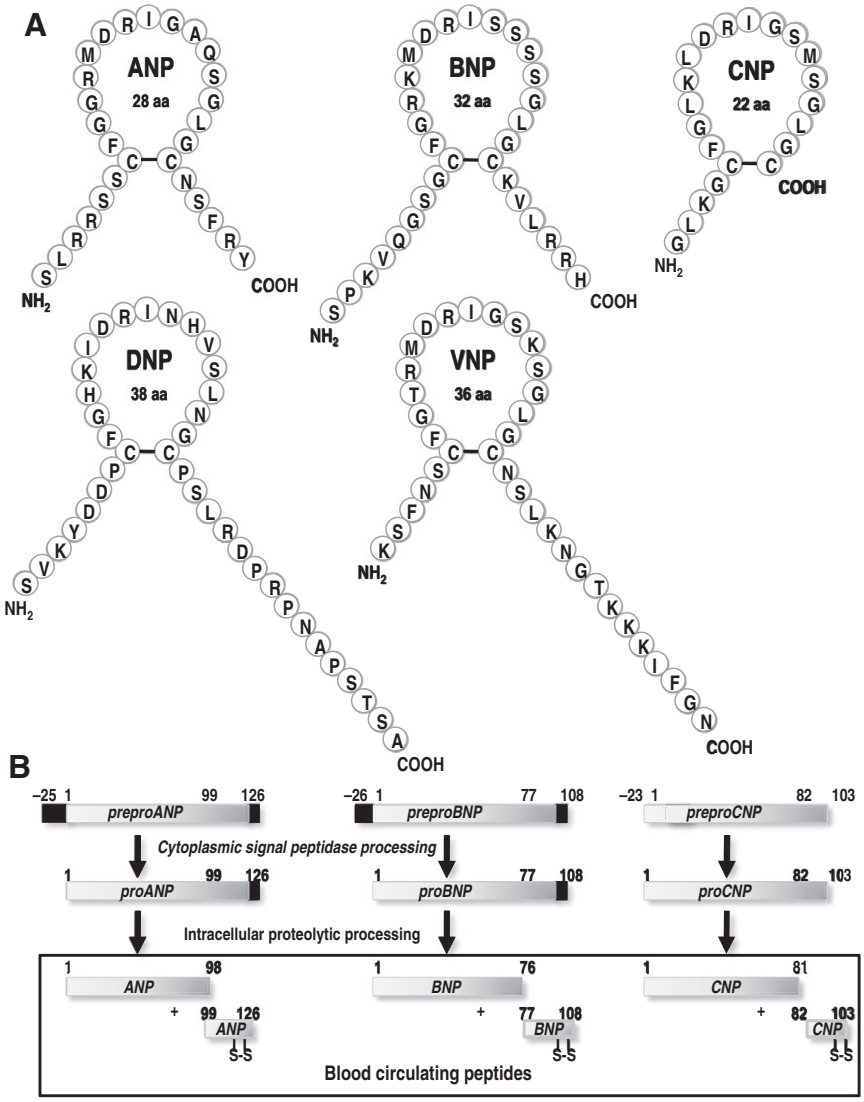


Fig. 2. (A) Sequences and structures of atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), C-type natriuretic peptide (CNP), ventricular natriuretic peptide (VNP) and *Dendroaspis* natriuretic peptide (DNP). The 17-amino-acid loop is indicated. (B) Schematic representation of the processing steps involved in the generation of the circulating forms of ANP, BNP and CNP from their precursors. Modified from Takei and Hirose (Takei and Hirose, 2002) and McGrath and de Bold (McGrath and de Bold, 2005).

affinities for ANP, BNP and CNP. NPRB is the natural receptor for CNP, with a high affinity also for the piscine VNP (Koller et al., 1991). NPRC and NPRD are GC-deficient. NPRC is able to bind all NP with high affinity and is mainly responsible for their clearance and degradation. NPRD shows a tetrameric structure like NPRA and NPRB but its functions remain to be elucidated [for references, see Takei and Hirose (Takei and Hirose, 2002)]. The NPRA cDNA and gene have been identified in mammals (e.g. human, rat, mouse), in teleosts (e.g. eel, trout, medaka) and in bullfrog (Garg and Pandey, 2005). NPRB is expressed in hagfish, eel, spiny dogfish, amphibians and mammals [for references, see Takei and Hirose (Takei and Hirose, 2002) and Takei et al. (Takei et al., 2007)]. NPRC is also present in both non-mammalian [e.g. elasmobranchs, bony fish, amphibians and turtle (Toop and Donald, 2004)] and mammalian vertebrates. So far, NPRD has only been found in eel (Kashiwagi et al., 1995).

Extensive genetic and molecular studies, mainly performed in fish, document the evolutionary pressure to conserve and, at the same time, diversify the primary structure of individual domains in precursor molecules and biosynthetic pathways. Conceivably, NP diversification may have accompanied the transition from aquatic to terrestrial environments, contributing to the consequent homeostatic cardiovascular and body fluid rearrangement (Inoue et al., 2003a; Takei et al., 2006). The diuretic, natriuretic and cardiovascular properties of NP improved animal fitness to cope with these dramatic changes, as experienced by many fish species in which a highly differentiated NP system may have contributed to body fluid homeostasis during the colonization of different habitats. Accordingly, fish have the most complex NP system, comprising at least four ligands and four receptors. By contrast, in tetrapods, the complete water-to-land transition, with the consequent need of water and sodium retention for body fluid regulation, permitted the disappearance (and/or substitution) of NP and NPR molecules (VNP *vs* DNP, and NPRD) of only vestigial significance, so that in mammals four hormones and three receptors are present (Inoue et al., 2003a).

A lesson arising from the fact that fish NP are more diverse than tetrapod NP may be that the immediate demands of the current environment shape the animal design together with its biomolecular regulatory systems, so that each animal achieves an equally optimal (or nearly optimal) match of components-to-performance-to-environmental challenges. This lesson contrasts the remnants of the still-lingering pre-Darwinian idea that views animals as '*progressive improvements driven by anticipation of a better*

Table 2. Natriuretic peptides in living organisms

Organism	Species	Peptide
ANIMALIA		
Vertebrata		
Mammalia	Several species (i.e. human, dog, mouse, rat, sheep, cow, pig, dromedary, dolphin, finback whale)	ANP, BNP, CNP
Aves	Chicken (*Gallus gallus*), domestic pigeon (*Columba livia*)	BNP, CNP, RNP
Reptilia		
Squamata	*Dendroaspis angusticeps* (venom)	DNP
Chelodinia	Long-necked tortoise (*Chelodina longicollis*)	ANP, BNP
Amphibia		
Anura	Bullfrog (*Rana catesbeiana*), toad (*Bufo marinus*), African clawed frog (*Xenopus laevis*)	ANP, BNP, CNP
Caudata	Newt (*Cynops pyrrhogaster*)	ANP, BNP
Osteichthyes		
Teleostei	Tilapia (*Oreochromis mossambicus*)	ANP, BNP
	Pufferfish (*Takifugu rubripes*)	ANP, BNP, CNP
	Medaka (*Oryzias latipes*)	BNP, CNP
	Eel (*Anguilla anguilla japonica*), rainbow trout (*Oncorhynchus mykiss*), coho salmon (*Oncorhynchus keta*)	ANP, VNP
Chondrostei	Sturgeon (*Acipenser transmontanum*)	ANP, BNP, CNP, VNP
Chondrichthyes		
Elasmobranchii	Japanese dogfish (*Triakis shyllia*), shark (*Squalus acanthias*), dogfish (*Scyliorhinus canicula*)	CNP
Cyclostomata	Hagfish (*Eptatretus burgeri*)	EbuNP
Invertebrata		
Secernentea	Roundworm (*Ascaris suum*)	ANP-like
Insecta	Silkworm (*Bombyx mori*)	
Crustacea	Blue crab (*Callinectes sapidus*)	
Bivalvia	Oyster (*Crassostrea virginica*)	
PROTISTA		
Ciliata	*Paramecium multimicronucleatum*	ANP-like
PLANTAE	*Dracena godseffiana*, *Metasequoia*, *Hedera helix*, *Zea mays*	ANP-like

For references, see text.
ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; CNP, C-type natriuretic peptide; DNP, *Dendroaspis* natriuretic peptide; EbuNP, *Eptatretus burgeri* NP; VNP, ventricular natriuretic peptide.

tomorrow' (Kardong, 1995), which is the old Lamarckian concept of the 'scale of nature' (*scala naturae*).

NP receptors: a hallmark of myocardial heterogeneity

The heart is the major site of synthesis and release of ANP, BNP and, when present in teleosts, VNP. CNP is predominantly an endothelial hormone; however, in eel, trout and elasmobranchs (i.e. *Scyliorhinus canicula*, *Triakis shyllia*), it is also a cardiac product (see Loretz and Pollina, 2000; Inoue et al., 2003b). Presumably, in elasmobranchs, a contribution to cardiac CNP production derives from the large endothelial surface of the well-developed arterial and venous coronary supply [see Tota (Tota, 1989) for references and comments].

The regional expression of cardiac NP provides the endocrine hallmark for the morphofunctional heterogeneity of the vertebrate heart (see Aardal and Helle, 1991). In adult non-mammalian vertebrates, from teleosts to reptiles, the whole heart synthesizes and releases NP (Reinecke et al., 1985; Netchitailo et al., 1986; Netchitailo et al., 1988; Donald et al., 1992; Fukuzawa et al., 1996; Loretz et al., 1997). This diffuse cardiac NP production, which is detectable in the ancient African lungfish (Larsen et al., 1994; Masini et al., 1996), is considered a very old acquisition of the heart [for references, see Takei et al. (Takei et al., 2006)]. Comparative analyses have revealed a different atrial and ventricular NP expression. Atrial NP production always exceeds that of the ventricle. When two atria are present (i.e. Amphibia), NP are present more in the left than in the right atrium (Netchitailo et al., 1986; Netchitailo et al., 1988; Feuilloley et al., 1993). If ANP and BNP are separately analyzed, this endocrine heterogeneity is even more evident. Examples from different species, including mammals, indicate that the atria contain more ANP than BNP, while BNP is the major ventricular product (Kasuya et al., 1992) [see McGrath et al. (McGrath et al., 2005) for references]. Interestingly, the topography of NP production correlates with the hemodynamic gradients and the sensor ability of the various cardiac chambers. NP richly populate the low-pressure venous regions of the heart, such as the portal vein heart of cyclostomes, the entirely venous heart of fish, the postcaval vein, the sinus venosus and the trabeculated atrial and septal regions of amphibians (Kasuya et al., 1992). In mammals, the highest NP production occurs at the level of the superior and inferior vena cava, the extrapulmonary veins and, more abundantly, the right atrium [see Aardal and Helle (Aardal and Helle, 1991) and references therein]. Likely, these strategic locations potentiate the sensor functions of the venous regions of the heart. The best example is the atrium, which, thanks to a rich pool of stretch-sensitive ion channels, instantaneously

monitors volume changes of the venous-return and consequently activates NP release.

Like their ligands, NPR are non-homogeneously expressed in the heart. A large survey on a variety of vertebrates, including several fish species, the frog and the quail, indicated that the various cardiac regions have different binding densities and affinities for homologous and heterologous ligands. Major binding sites are located in the atrium and ventricle and in the outflow tract, such as the bulbus arteriosus, of fish and amphibians (Cerra et al., 1992; Cerra et al., 1993; Cerra et al., 1996). Application of homologous peptides for radioreceptor analysis identified different types of NPR (Cerra et al., 1996). In the eel, NPRC-like receptors are present in atrial and ventricular myocardium, while the ventricular EE appears to express an NPRA-type able to bind ANP and VNP with almost equal affinity. Moreover, a receptor with high CNP affinity, presumably NPRB, is expressed in the bulbus arteriosus, where it may mediate a CNP-dependent modulation of the bulbar hemodynamics (e.g. the Windkessel function) that allows depulsation of the large systolic oscillations in blood pressure and blood flow (see Icardo et al., 2000).

Such zonal distribution of cardiac NPR is maintained in mammals in which atria, ventricles, coronaries and aorta express three NPR-encoding genes and transcripts. NPRA is the major functional NPR in atrial and ventricular myocardiocytes, while NPRB is mostly expressed in non-myocytes, including ventricular EE (Kim et al., 1999), but also seems to be present in atrial myocytes (Doyle et al., 2002). Ventricular NPR distribution is not homogeneous, the right ventricular EE showing the major receptor location with respect to the left ventricular EE and the myocardium of both ventricles. Moreover, fewer receptors are present in the septal side than in the free wall of the left ventricular EE (Kim et al., 1999). This compartmentalization provides the various heart regions with different abilities to bind NP and is essential for normal cardiac function, as regional alterations of NPR expression associate with a deteriorated heart performance [for references, see Cerra and Pellegrino (Cerra and Pellegrino, 2007)]. For example, NPR expressed in the ventricular EE (Wilcox et al., 1991; Rutherford et al., 1992) disappear in the presence of right ventricular hypertrophy (Kim et al., 1999), impairing EE–myocardium communications and thereby affecting contractility and contributing to cardiac damage.

NP in heart–vessel and ion–water homeostasis

It is generally accepted that in all vertebrates the primary acute stimulus for atrial NP release is the stretch induced by hypervolemia (Ruskohao, 1992). Once released, NP elicit endocrine/paracrine/autocrine actions on a large number of proximal and distal targets, generating a complex multilevel network that controls heart–vessel and blood volume–ion homeostasis [for references, see Cerra and Pellegrino (Cerra and Pellegrino, 2007)]. This network orchestrates direct chronotropic, inotropic and potent vasorelaxant responses and indirect actions that minimize cardiac hemodynamic loads. Unloading is obtained *via* diuretic and natriuretic mechanisms, as well as *via* a reduction of sodium and water content attained through a central inhibition of thirst and sodium appetite in conjunction with an intestinal decrease of water and sodium absorption. NP also depress aldosterone and vasopressin secretion, thus further promoting renal water and sodium excretion. As the end point, hypervolemia is faced and blood volume is rapidly restored to normal (reviewed in Takei and Hirose, 2002). However, the strong interdependence between osmoregulation and cardiovascular homeostasis, together with the complexity of these circuits, has recently questioned not only the paradigm of hypervolemia-elicited NP release but also whether this hormonal system is mainly a heart–vessel or an ion–fluid regulator. The question is of obvious importance not only for a more satisfactory interpretation of basic homeostatic mechanisms but also for a better evaluation of the diagnostic and therapeutic roles currently assigned to NP in human pathologies.

Osmoregulatory *versus* cardiovascular: not necessarily alternatives

In fish, more than in other vertebrates, the major control of heart performance is achieved through filling pressure and volume loading, i.e. the Frank–Starling mechanism. In these animals, blood volume is strongly influenced by the osmotic gradient existing with the surrounding water. Fish osmoregulate to maintain body fluid volume and concentration, since water enters and salts leave the body in freshwater environments, while the opposite is true in seawater (Karnaky, 1998). Moreover, due to their adaptability to different environments (from euryhalinity to stenohalinity), fish represent an extraordinarily rich field to separate cardiovascular from ion–fluid NP functions. Here, we will summarize two hypotheses (reviewed by Johnson and Olson, 2008; Johnson and Olson, 2009a; Johnson and Olson, 2009b) that have been proposed to clarify this issue.

The first hypothesis, based essentially on a few studies on the euryhaline Japanese eel, points to NP as a major osmoregulatory system; this hypothesis later adopted and refined a new cardioprotective function in tetrapods (Takei and Hirose, 2002; Loretz and Pollina, 2000; Takei and Hirose, 2002; Takei et al., 2006; Takei et al., 2007; Tsukada and Takei, 2006). In fish, hyperosmolality contributes importantly to cardiac NP release. In eel, freshwater-to-seawater transfer increases blood osmolarity while decreasing blood volume and is considered the most potent stimulus of ANP and VNP (Kaiya and Takei, 1996a; Kaiya and Takei, 1996b). Presumably, the increased plasma Na^+ levels, consequent to seawater transfer, increase ANP secretion, and thus the major ANP target is not blood volume but plasma Na^+. Moreover, in seawater eels, ANP is antidipsogenic but not hypotensive (Tsuchida and Takei, 1998). All these observations indicate a situation which is opposite to that found in mammals, in which the decrease in blood volume inhibits ANP secretion and the ANP-dependent hemodynamic actions appear dominant with respect to osmoregulatory effects.

The second hypothesis points to NP as major cardioprotective regulators that, by depleting blood volume, protect the heart from excessive preload and afterload (Olson et al., 1997). As indicated by direct and indirect evidence obtained in various species, this hypothesis is supported by the ubiquitous cardiac NP expression in vertebrates (regardless of their osmoregulatory features), their common vasorelaxing action and the universal stretch-induced myocardial release of NP (Johnson and Olson, 2008). In rainbow trout, cardiac NP and cardiovascular NPR respond principally to volume, not salt overload (Johnson and Olson, 2009a). In fact, in this teleost, the increased filling pressure potently stimulates cardiac NP secretion (Cousins and Farrell, 1996). Once released, NP relax both arterial and venous branchial vessels, thus reducing gill perfusion pressures and resistances (see Farrell and Olson, 2000). This downstream effect prevents the excessive hemodynamic load imposed on the heart by elevated afterloads occurring beyond the Windkessel control exerted by the bulbus arteriosus. Being additional and/or alternative to the Starling response, it significantly contributes to regulate piscine heart performance. Therefore, the increased compliance and the

decreased vascular tone of the small veins (both reducing venous-return) and the branchial vasculature are viewed as the two most prominent cardiovascular actions of NP. When venous filling pressure increases, a stretch-induced stimulation activates the afferent limb of NP regulation, the consequent increased NP release from the heart providing an effective control system for regulating mean circulatory filling pressure and therefore venous-return (Olson et al., 1997). Interestingly, in freshwater fish, which experience only volume and not salt load, NP are constitutively released (trout) (Olson and Duff, 1992). This suggests that the osmoregulatory hypothesis cannot fully explain the presence of the NP system in freshwater teleosts. Conceivably, in the cardiovascular system, NP appear better designed as volume than salt regulators (Johnson and Olson, 2008; Johnson and Olson, 2009a). Therefore, according to Johnson and Olson (Johnson and Olson, 2009b), all the above observations point to a fundamental similarity of piscine and mammalian NP systems in protecting the heart from volume overload.

However, rather than being alternatives, these hypotheses may represent two integrated parts of a whole general homeostatic framework orchestrated by NP. Several pieces of evidence point to a unifying synthesis. For example, a functional link between hemodynamic and NP-dependent osmotic mechanisms has been suggested in elasmobranchs, in which a relationship between volume increase, salt overload, CNP production and rectal gland activation has been demonstrated (Solomon et al., 1984; Gunning et al., 1997; Silva et al., 1999; Olson, 1999). Moreover, the NP-dependent reduction in gill perfusion may produce indirect effects on branchial ion transport, thus changing blood Na^+ and Cl^- concentrations and osmolality (see Toop and Donald, 2004; Evans, 2002).

Because of their biphasic life style and consequently remarkable hydro-osmotic challenges, it may be expected that amphibians have pressurized NP diversification towards a tighter integration of their osmoregulatory and/or cardiovascular properties. The amphibian heart is very sensitive to stretch-mediated mechanisms, and NP may contribute to improve the cardiac functional plasticity in the presence of the hydration/dehydration events. So far, limited evidence indicates that the amphibian heart is sensitive to NP. For example, on the isolated atria of *Rana tigrina* (Chiu and Lee, 1992) and on the isolated and working heart of *Rana esculenta* (Cerra et al., 2003), ANP induces negative chronotropic and inotropic effects. In *Rana esculenta* heart, these effects are presumably mediated by two classes of high- and low-affinity NP binding sites (NPRA/NPRB) detected in both the ventricular EE and myocardium (Cerra et al., 2003). In fact, while the negative inotropism induced by frog ANF-(1–24) was unchanged by the soluble GC inhibitor ODQ, it was abolished by the competitive GC-coupled-NPR receptor antagonist anantin, indicating an involvement of pGC. This was also confirmed by the frog ANF-(1–24)-dependent negative inotropism, which appeared to be independent of the functional integrity of EE (Cerra et al., 2003).

NP from ontogeny to physio-pathology: the mammalian lesson

During the past 20 years, an impressive effort has been made in many mammalian natural and genetically modified models to understand the basic biology and physiopathology of NP, including putative targets for diagnosis and therapy in humans. An important task was the analysis of the ontogenetic development of cardiac NP. Starting from the very early stages of ontogenesis (i.e. beginning at day 9 to a peak at day 15 of gestation), both atria and ventricles express ANP and BNP but not CNP. ANP is one of the first hormones produced by mesodermal derivatives (Thompson et al., 1986; Scott and Jennes, 1988). These stages are landmarks of cardiac development, day 9 being the beginning of the regular beating, day 12 being the progressive septation and formation of the four chambers, and day 15 being the initial alteration of the heart axis. As the development of the cardiac myoendocrine NP system is strongly conditioned by surrounding tissues, for example the endoderm (Ruiz et al., 1995), NP represent important markers of normal cardiac development. During fetal life, both volume loading and osmolarity trigger NP release. As in adults, these peptides elicit vasorelaxant and hypotensive actions in both fetus and placenta; moreover, they control cell growth and proliferation, thus participating in cardiac organogenesis [for references, see Cameron and Ellmers (Cameron and Ellmers, 2003)]. After birth, ventricular NP gene transcription tends to decline so that in the normal adult mammalian heart the atria are the main sites of synthesis for both ANP and BNP, while the ventricle retains a low BNP synthetic ability [for references, see D'Souza et al. (D'Souza et al., 2004)]. By contrast, CNP is produced in very limited amounts in the heart (Ahluwalia et al., 2004).

In healthy adult mammals, a rapid increase in cardiac NP release is encountered under all physiological conditions that increase venous-return, such as physical exercise, head-out water immersion and rapid changes from standing to supine position. Stretch-secretion coupling represents the major activator of the NP system. An increased cardiac rhythm also stimulates NP secretion, so that, conceivably, the connection between impulse conducting and contractile cardiac cells may contribute to switching on the endocrine heart (Qi et al., 2000). However, hydration/dehydration-dependent processes may also play a role, as suggested by the fluctuations in cardiac ANP content and plasma levels experienced by water-deficiency-tolerant mammals. In the desert rat (Lacas et al., 1998; Lacas et al., 2000) and the dromedary camel (Osman et al., 2004), NP-dependent mechanisms significantly contribute to coping with these environmental challenges. Notably, in the camel, intensive rehydration after a long period of water deprivation does not increase blood volume, the water being first stored in the rumen-like forestomach and then directed to the interstitium. Accordingly, they represent well-suited organisms to analyze hypervolemia *vs* osmolarity as triggers for NP release.

ANP, BNP and CNP negatively affect cardiac contractility. However, conflicting and confusing data have been reported on the inotropic potency of NP. In particular, depending on different experimental models employed, ANP and CNP appear poorly effective or ineffective in contractility (see D'Souza et al., 2004). *In vitro vs in vivo* preparations or different peptide concentrations may explain the inconsistency of the results. For example, low NP concentrations may generate low amounts of cGMP, which induce positive inotropic effects *via* either PKA activation (Kojda and Kottenberg, 1999) or ryanodyne receptors and L-type Ca^{2+} channel stimulation (Massion and Balligand, 2003). By contrast, higher peptide concentrations may reduce contraction through PKG-dependent inhibition of Ca^{2+} channels (Massion and Balligand, 2003) and a reduction of myofilament sensitivity to Ca^{2+} through troponin I phosphorylation (Shah, 1996). Note that, as outlined below, these NP-dependent signal-transduction pathways utilize intracellular effector components that often counteract the effects elicited by CA-dependent cascades.

Distal from the heart, NP promote diuresis, natriuresis and vasodilation. They decrease shear stress and modulate coagulation and fibrinolysis, thus preventing endothelial dysfunction. They also inhibit platelet activation, inflammation and abnormal growth, an

action mainly exerted by CNP, which appears to counterbalance vascular remodeling [for references, see Cerra and Pellegrino (Cerra and Pellegrino, 2007)]. All these actions are exerted in concert with NO, prostaglandins and other vasodilator peptides and contribute to counteract the sodium-retaining, vasoconstrictive, thrombophylic, proinflammatory and prohypertrophic actions elicited by other stress-activated hormonal systems, such as the RAS. Under physiological conditions, NP and RAS are well equilibrated by feedback mechanisms. In the presence of some cardiovascular diseases, NP become predominant and initiate a compensatory mechanism that may lead progressively to detrimental effects. Of note, the altered topography of NP production represents a first and important sign of these changes. In the adult mammalian heart, under cardiac overload, hypertrophy, hypoxia and sustained adrenergic stimulation, BNP expression strongly increases; the ventricle progressively becomes the major site for NP production, while ANP production undergoes only slight changes [for references, see de Bold et al. (de Bold et al., 2001)]. This shift from atrial to ventricular production changes the normal plasma concentration ratio of ANP to BNP and makes BNP an important indicator of pathological myocyte re-differentiation with the characteristic of an immediate-response gene (Mäntymaa et al., 1993; Magga et al., 1997). As a consequence, BNP has now been adopted as a diagnostic and prognostic marker of ventricular dysfunction (de Bold et al., 2001). Interestingly, in hypertrophic cardiomyocytes and in failing myocardium post-infarction, the increased expression of corin (Tran et al., 2004), the enzyme responsible for post-translational processing of both proANP and proBNP, provides the molecular basis for the augmented pathological production of NP.

To clarify all the NP-related pathological implications, a large number of investigations have been performed during the past 20 years, and their results are summarized in excellent reviews to which the reader may refer to (D'Souza et al., 2004; McGrath et al., 2005). As a biomedically relevant outcome, NP emerged as potent pharmacological agents currently used for acute decompensation in heart failure; i.e. BNP (Nerisitide®) (Keating and Goa, 2003) and ANF (Carperitide®) (Kitashiro et al., 1999).

NP, CA and the stressed heart

Since the very first studies on NP, the co-existence of ANP-like immunoreactivity and CA in the secretion granules of the adrenal chromaffin and the myoendocrine cells of cyclostomes (Reinecke et al., 1987) and in adrenal chromaffin cells of bony fish, amphibians, reptiles, birds and mammals (Reinecke et al., 1992; Wolfensberger et al., 1995; Takei et al., 1997) strongly suggested that, in vertebrates, the two systems have evolved together. As functional counterparts of this morphological relationship, in many vertebrates, including mammals, the same stimulus (i.e. ACh-mediated nicotinic activation) stimulates the release of both NP (ANP, BNP and CNP) and CA (Okazaki et al., 1989; Nguyen et al., 1990; Babinski et al., 1992).

Several lines of evidence now support the view that NP represent an endocrine counteraction of the major excitatory limbs of the stress system, i.e. RAS and CA. Indeed, through the synthesis/release of NP, the vertebrate heart activates endocrine feedbacks to counterbalance the effects induced by a variety of stressors (i.e. ET-1, ANG II, glucocorticoids, sex steroid hormones, thyroid hormones, some growth factors, and cytokines) [for references, see Clerico et al. (Clerico et al., 2006)]. NP and CA are under reciprocal influence. This was indicated, for example, by the control of the granule bioactivity exerted through NP-induced activation of chromaffin cell NPR, mainly NPRA (Kloas et al., 1994; Grandclément et al., 1997). In non-mammalian vertebrates, NP elevate circulating CA by mechanisms that are still poorly understood, including, in elasmobranchs, an involvement of nicotinic receptors (Montpetit et al., 2001). In *S. acanthias*, administration of homologous CNP increases CA plasma levels, although a direct *in situ* effect on chromaffin cells was not observed. The increased circulating CA might contribute to counterbalancing the CNP-induced hypotension (Montpetit et al., 2001), which is of importance for preserving the cardiovascular function of this animal, which lacks organized sympathetic nervous control (Nilsson et al., 1975). In teleosts, NPs and CAs are also functionally associated. This is indicated by an increased release of CA from chromaffin cells and/or SNS activation following administration of ANP and VNP (Olson and Duff, 1992; McKendry et al., 1999). Presumably, NP directly act on the chromaffin tissue thanks to the presence of NP binding sites (Kloas et al., 1994). It cannot be excluded that SNS activation is secondary to NP-mediated hypotension.

CA contribute to the cross-talk with NP by stimulating their cardiac biosynthetic pathways. In *Rana ridibunda*, exogenous ISO and adrenaline activate p38-MAPK, which increases ventricular expression of ANP (Aggeli et al., 2002). In the isolated atria of *R. tigrina*, ANP counteracts the effects of β-ARs stimulation (Chiu and Lee, 1992). In relation to the CA-induced toxicity (see above), the cardioprotection elicited by NP may be of great importance. In fact, lower experimental temperatures, which increase myocardial resistance to CA-dependent necrosis (Carlsten et al., 1983b; Volkmann, 1985; Herman et al., 1986), also increase ISO-stimulated p38-MAPK activation and ANP production (Aggeli et al., 2002).

In mammals, the NP–CA interactions show somewhat different patterns. Current data point to a sympatholytic action elicited by NP, particularly ANP. In both adrenal gland and SNS nerve terminals, NP reduce CA production *via* a cGMP-dependent activation of tyrosine hydroxylase, the rate-limiting enzyme in CA synthesis (Holtz et al., 1987). This effect is corroborated by the high circulating CA levels found in genetically modified mice with ANP gene disruption (Melo et al., 1999). On the other hand, the effects of adrenergic stimulation on mammalian cardiac NP release are still in part unknown because of confusing results mainly derived from clinical studies carried out in the presence of different pathological conditions and/or therapies. It was found that α-ARs activation enhances the expression of NP, while ISO reduced BNP, but not ANP, atrial secretion (Yuan et al., 2009). Furthermore, hypertensive patients treated with β-blockers, show increased plasma levels of ANP and/or BNP, while in congestive heart failure, chronic treatment with β-blockers, which improves cardiac function and reduces cardiac filling pressure and volumes, is usually associated with a significant BNP reduction (see for ref. Clerico et al., 2006). However, whether these effects on NP production and release are due to a direct β-ARs-specific action or to the loss of the stimulus for BNP secretion due to the reduction in cardiac filling pressure and volume remains a task for future investigations.

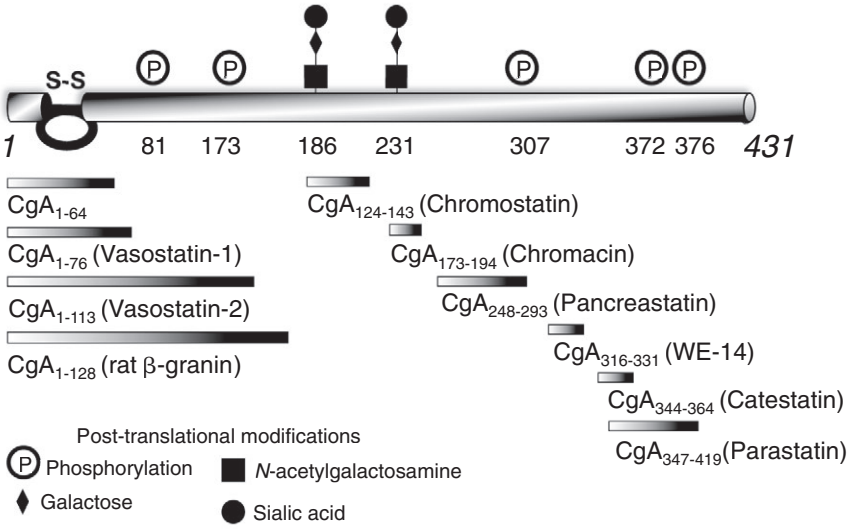
On the whole, despite the different mechanisms that may operate in mammals *vs* fish and amphibians, the available evidence points to NP and CA as major interacting components of a powerful counter-regulatory system. While, in mammals, NP may blunt the adrenergic stress by acting predominantly at its very first level, i.e. by reducing the synthesis of CA, in fish and amphibia NP may act predominantly at the distal level, by counterbalancing the

adrenergic-dependent stress on target organs, initially the heart. This raises the following challenging questions: (1) do the 'fish/amphibian' and 'mammalian' patterns represent two examples of different strategies evolved during vertebrate phylogeny; (2) do these different patterns basically result from differences in the CA/SNS arrangement and/or the mechanisms for blood pressure and volume regulation; and (iii) to what extent have these counter-regulatory patterns, which integrate extrinsic and intrinsic cardiac excitatory and inhibitory stimuli, been shaped by selective pressure to protect the stressed heart?

Chromogranin A

A prohormone constituent of the CA-containing granules

The first evidence of a role for chromogranin A (CgA) came in the mid-1960s with the discovery that this protein is the major soluble constituent of the secretory granules of the chromaffin cells of the bovine adrenal medulla, where it is co-stored and, upon cholinergic stimulation, co-released with CA (Banks and Helle, 1965; Blaschko et al., 1967). CgA is a member of the granin family, which includes chromogranin B and secretogranin II. Genetically distinct and uniquely acidic proteins, the three granins are co-stored with amines and peptide hormones in species-, organ- and tissue-specific patterns. They are present in the nervous and immune systems as well as in the diffuse neuroendocrine system in different locations throughout the body, e.g. the adrenal medulla, the parathyroid gland, the gastro-entero-pancreatic system and the heart itself. Immunoreactive evidence detected CgA-like proteins in the nervous system of many invertebrates including coelenterates [CgA-derived fragment WE-14 (Barkatullah et al., 1997)], the nematode parasite *Ascaris suum* [the hexapeptide KGQELE, flanking the C-terminus of rat CgA250-301 (Smart et al., 1992)] and within the secretory granules (trichocystis) of the protozoan *Parameciun tetraurelia* (Peterson et al., 1987). CgA is ubiquitous and highly conserved throughout vertebrates (Tota et al., 2007a; Helle et al., 2007), being present in teleost fish [the coho salmon, *Oncorhynchus kisutch* (Deftos et al., 1987)], amphibians [the frog *R. ridibunda* (Reinecke et al., 1991)], reptiles [the lizard *Lacerta viridis* (Trandaburu et al., 1999)], birds [the quail *Coturnix coturnix japonica* (Reinecke et al., 1991)], humans, pigs and rats. The very high interspecies homology points to the long evolutionary history of CgA. Among mammals, only minor differences in primary sequence and post-translational modifications have been documented, as exemplified by the bovine protein ($bCgA_{1-431}$), which is shorter than the human ($hCgA_{1-439}$) and rat proteins ($rCgA_{1-448}$) (Tota et al., 2007a; Helle et al., 2007). In the mid-1980s, following the breakthrough of cDNA technology and the prohormone concept introduced by Eiden (Eiden, 1987), CgA was identified as a prohormone precursor of many fragments, generated by tissue-specific proteolytic cleavage operated by several proteases and pro-hormone convertases (e.g. PC1/3 and PC2 co-stored in the neurosecretory granules) on multiple pairs of dibasic sites lying along the CgA sequence (Fig. 3) [see Helle et al. (Helle et al., 2007) for extensive references]. The finding that the N- and C-terminal domains, CgA_{1-76} and $CgA_{316-431}$, are highly conserved

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Human     LPVNSPMNKGDTEVMKCIVEVISDTLSKPSPMPVSQECFETLRGD
Rat       LPVNSPMTKGDTKVMKCVLEVISDSLSKPSPMPVSPECLETLQGD
Frog      LPVSSLEGEDNTKVMKCIVEVISDTLSKPNPVPITQDCLETLRGD
Bovine    LPVNSPMNKGDTEVMKCIVEVISDTLSKPSPMPVSKECFETLRGD
                          17                  38

Human     ERILSILRHQNLLKELQDLALQGAKERA----------------HQ
Rat       ERVLSILRHQNLLKELQDLALQGAKERAQQQQQQQQQQQQQQQQ-
Frog      ERIISILRHQNLLKELQELAAQGAMERLQKA------------HQ
Bovine    ERILSILRHQNLLKELQDLALQGAKERAT--------------HQ

Human     QKKHSGFEDELSEVLENQSSQAELKEAVEEPSSKDVMEKR
Rat       QQQHSSFEDELSEVFENQSPAAKHGDAASEAPSKDTVEKR
Frog      QKKNRGYEEELSGVLEKQDDKAPVSEKSDEFRLHSKFSES
Bovine    QKKHSSYEDELSEVLEKPNDQAEPKEVTEEVSSKDAAEKR
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Fig. 3. (A) Chromogranin A (CgA) and its derived peptides. The N-terminal disulphide bridge and the post-translational modifications of the bovine sequence are indicated (modified from Tota et al., 2007a). (B) Sequence alignment of the N-terminal region (1–115) of human, rat, frog and bovine CgA, showing the high degree of homology and the two cysteines involved in the disulphide bridge formation. Modified from Turquier et al. (Turquier et al., 1999), Lugardon et al. (Lugardon et al., 2000) and Cerra et al. (Cerra et al., 2008).

sequences in mammals is consistent with the role of CgA functioning as a prohormone for shorter regulatory peptides. The endo-proteolytic process generates several peptides of biological importance, including the dysglycemic hormone pancreastatin (Tatemoto et al., 1986), the vasodilator vasostatin 1 (VS-1) (Aardal et al., 1993) and the catecholamine release inhibitory peptide catestatin (Cts) [human $CgA_{352-372}$, bovine $CgA_{344-364}$ (Mahata et al., 1997; Mahata et al., 2003; Mahata et al., 2004)]. Cts and pancreastatin have been postulated as important counter-regulatory hormones in 'zero steady-state error' homeostasis (Koeslag et al., 1999). This concept is based on pairs of counter-regulatory hormones, which operate as an 'integral' controller, bringing the controlled variable back to the 'set point' at any steady-state disturbance; i.e. the equilibrium is finely regulated by the balance between two hormones. In this context, we will highlight the cardiovascular properties of CgA, VS-1 and Cts in an attempt to provide new insights and perspectives in cardiac biology and physiopathology.

The importance of CgA in cardiac physiology and physiopathology

Evidence from the past decade suggests that CgA and CgA-derived VS-1 and Cts are new players in the scenario of the endocrine heart. Detected since 1990 in the granules of rat atrial myoendocrine cells (Steiner et al., 1990) and in the cells of the cardiac conduction system co-localized with the α1E subunit of the voltage-gated calcium channel (Weiergraber et al., 2000), CgA was identified more recently in the human ventricular myocardium (Pieroni et al., 2007). Under normal conditions, CgA is expressed at low levels, only detectable by PCR and ELISA, but in the presence of dilated and hypertrophic cardiomyopathy, the peptide is also immunologically detectable on tissue sections (Pieroni et al., 2007). Importantly, in the heart, CgA correlates with the cardiac NP system, since in atrial myocytes and in the conduction cells, it is co-stored with ANP, while in the ventricle it co-localizes with BNP (Pieroni et al., 2007) (Fig. 4). In both decompensated and hypertrophic heart, increased plasma CgA levels parallel the increment of circulating BNP (Pieroni et al., 2007), strongly suggesting that the stretch-induced release and/or transcriptional up-regulation mechanisms described for NP can also be operative for cardiac CgA (Pieroni et al., 2007). The importance of CgA in human cardiovascular homeostasis is further documented by its increased plasma levels in various diseases [i.e. neuroendocrine tumours: Ceconi et al. (Ceconi et al., 2002)], as well as chronic heart failure (Nobels et al., 1994), and its over-expression in human dilated and hypertrophic cardiomyopathy (Pieroni et al., 2007). More recently, Jansson et al. demonstrated that circulating levels of CgA provide prognostic information (long-term mortality and heart failure hospitalization) independent of conventional risk markers in acute coronary syndromes (Jansson et al., 2009). Moreover, genetic ablation of the chromogranin A (*Chga*) gene causes high blood pressure in mice, which can be rescued by the introduction of the human *CHGA* gene in the $Chga^{-/-}$ background (Mahapatra et al., 2005). Of note, plasma concentrations (~1.5 nmol l^{-1}) of the CgA-derived fragment Cts decrease in normotensive subjects with a family history of hypertension and increased epinephrine secretion; this is particularly evident in patients with essential hypertension, i.e. the complex chronic disorder with a poorly understood pathogenesis (O'Connor et al., 2002).

The CgA-derived vasostatins: novel cardiac stabilizers

VS-1 corresponds to the highly conserved vertebrate domain CgA_{1-76}, while VS-2 corresponds to the less conserved domain CgA_{1-113}. Named 'vasostatins' (VS) for their ability to relax vessels precontracted by high endothelin-1 (ET-1) and potassium concentrations (Aardal and Helle, 1992), they have been identified in both poikilotherm (frog) and homeotherm (rat, pig, bovine, human) vertebrates. The peptide fragments sequenced so far exhibit a very high percentage identity, since they share important traits such as the presence, in all species, of the sequence 50–62 (100% identity) and a disulfide bridge between C17 and C38 that appears crucial for their biological activity (Helle et al., 2007). Together with the other shorter VS peptides CgA_{1-40}, CgA_{4-57}, CgA_{47-66} and CgA_{67-76}, VS-1 and VS-2 are naturally generated within the matrix of chromaffin granules and are co-released with CA following chromaffin cell stimulation, for example by ACh (Metz-Boutigue et al., 1993). Recent evidence indicates the possible intracardiac production of VS. In fact, several N-terminal fragments containing the VS-1 domain were detected in rat heart extracts (CgA_{4-113}, CgA_{1-124}, CgA_{1-135} and CgA_{1-199}) together with a larger fragment, presumably corresponding to the intact CgA, suggesting an intracardiac cleavage of the precursor (Glattard et al., 2006).

In addition to their vasorelaxant properties, VS show many other autocrine, paracrine and/or endocrine functions [for references, see Helle et al. (Helle et al., 2007) and Tota et al. (Tota et al., 2007a)].

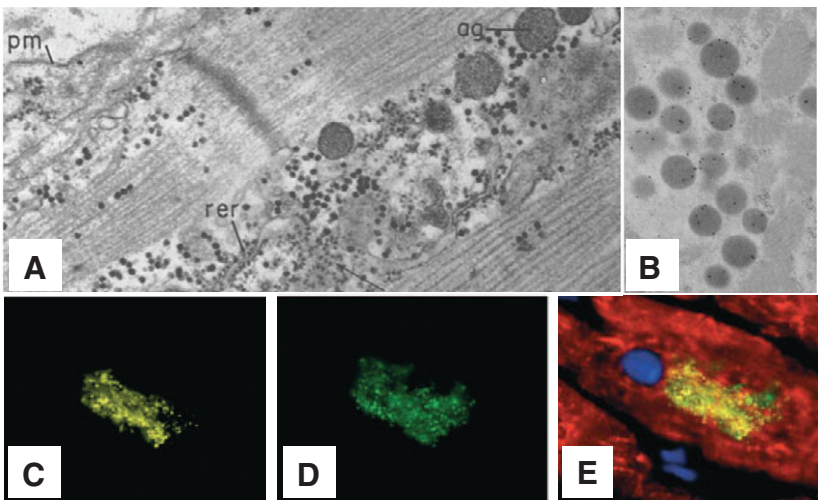

Fig. 4. Location of electron-dense granules in the mammalian heart. (A,B) rat atrium; (C–E) human ventricular myocytes. (A,B) Atrial granules decorated with immunogold particles labeled with antisera against N-terminal ANF fragments. (C) B-type natriuretic peptide (BNP) staining (yellow fluorescence); (D) Chromogranin A (CgA) staining (green fluorescence); (E) combination of BNP and CgA in myocyte cytoplasm; nuclei are in blue. Abbreviations: ag, atrial granules; pm, plasma membrane; rer, rough endoplasmic reticulum. For magnification, see original articles. Modified from (A) Jamieson and Palade (Jamieson and Palade, 1964); (B) Thibault et al. (Thibault et al., 1987); (C–E) Pieroni et al. (Pieroni et al., 2007).

According to their multifunctional role, it is not surprising that they contribute to the humoral regulation of the heart, as we demonstrated comparatively in both poikilotherm (eel, frog) and homeotherm (rat) vertebrates (Tota et al., 2007a). The major VS-induced actions consist of both negative inotropism (eel, frog, rat) and lusitropism (rat) and of the relevant counteraction of the β-ARs-mediated positive inotropism, typically induced by ISO (eel, frog, rat), achieved through a functional non-competitive type of antagonism (Fig. 5) (Tota et al., 2004; Imbrogno et al., 2004; Cerra et al., 2006; Pieroni et al., 2007). As detailed in the rat heart, these effects are independent of f_H and coronary performance and are comparable to those documented in the trabeculate and luminally supplied eel and frog hearts. Furthermore, using the isolated and perfused frog heart working at physiological loads as a bioassay for structure–function analyses, we demonstrated that the disulfide bridge is crucial for the marked negative inotropism whether mechanically activated or stimulated by ISO. By contrast, neither the N- nor the C-terminal group of VS-1 is critical for the cardiosuppressant effect of the peptide (Tota et al., 2003). Importantly, we demonstrated in the rat heart that VS-1 also exerts cardioprotection by reducing the effects of ischemia in a way that mimics ischemic preconditioning (Cappello et al., 2007). On the whole, the cardiotropic and vasoactive properties of CgA-derived VS, together with their ischemic preconditioning-like influence, suggest that these peptides function as homeostatic stabilizers of the cardiovascular system, particularly under conditions of stress (i.e. sympathetic overstimulation or cardiac injury).

Action mechanism(s) of VS

As the identification of classic high-affinity receptors remains elusive, the first upstream event of VS action is still enigmatic. However, alternative receptor-independent cell penetration (antimicrobial action) or cell microdomain perturbation (cardiac inotropism)-associated mechanisms were hypothesized (Helle et al., 2007; Tota et al., 2007a).

Studies on eel, frog and rat hearts indicated aspects of unity and diversity in the intracellular downstream cascades underlying the VS-induced cardiac actions. In eel and rat, but not frog, the VS-1-elicited negative inotropism involves β-ARs receptors, PTx-sensitive G-proteins and the NO–cGMP–PKG pathway. Only in the eel are cholinergic M1 receptors also involved. In both eel and frog, the VS-1-induced negative inotropism is also abolished by the inhibition of either potassium or calcium fluxes, emphasizing the relevance of spatially restricted membrane domains in which receptors, modulatory proteins and ion channels may be functionally coupled (Corti et al., 2004; Imbrogno et al., 2004; Cappello et al., 2007). As, in cardiomyocytes, the above signaling molecules are clustered with their substrates in specialized membrane microenvironments – the caveolae – these were proposed as the action sites of the peptide [for references, see Tota et al. (Tota et al., 2007a)]. Moreover, evidence in eel, frog and rat indicates that the cytoskeleton is involved in the functional coupling of VS-1 to its intracellular signaling partners [abolished in frog (Mazza et al., 2007) and in rat (Angelone et al., 2007; Angelone et al., 2009); severely reduced in eel (Mazza et al., 2007)].

The message coming from these comparative studies is that the VS-mediated negative inotropism in vertebrates involves different intracellular mechanisms. In this regard, the electrophysiological and fluorimetric confocal imaging investigations carried out by Gallo and co-workers on rat papillary muscles and bovine aortic endothelial cells (BAE-1) reveal the major involvement of the endothelium-produced NO and of phosphatidylinositol 3-kinase (PI3K) in transducing the cardiac effects of recombinant human VS-1 (hrSTA–CgA_{1-76}) (Gallo et al., 2007). Therefore, the anti-adrenergic effect elicited by VS-1 appears mainly due to a PI3K-dependent NO release by endothelial cells, rather than to a direct action on cardiomyocytes (Gallo et al., 2007). These data were further corroborated by a recent study (Cerra et al., 2008). In fact, the same endothelial PI3K-dependent NO release and the intracellular cGMP–PKG cascade were involved in the negative inotropism elicited in the rat heart by the shorter homologous VS fragment, rat CgA_{1-64} (Cerra et al., 2008). Of note, intact NO signaling is also necessary for the ischemic preconditioning-like protection induced by human recombinant VS-1 on the rat heart (Cappello et al., 2007).

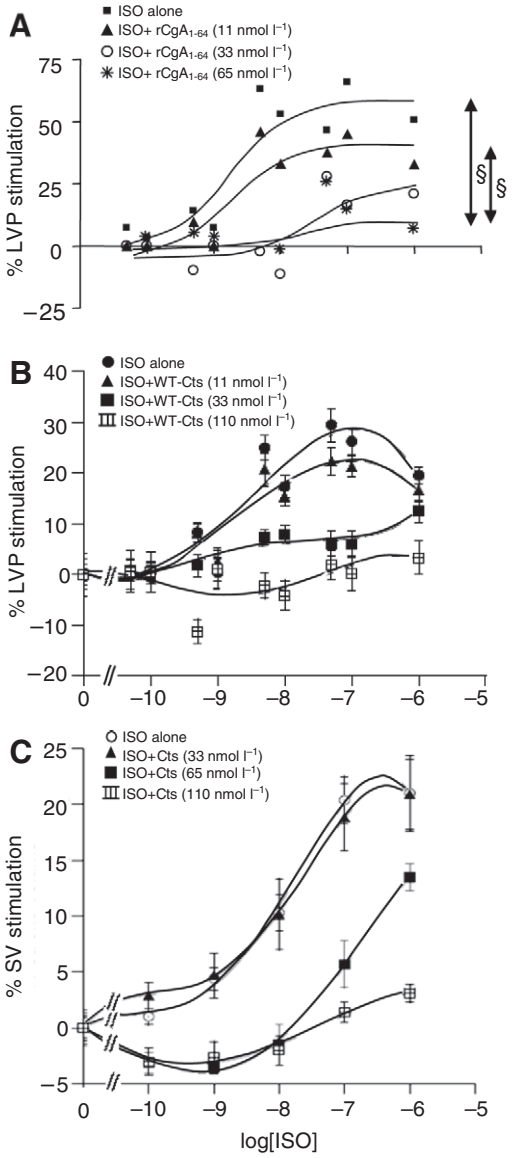

Fig. 5. Concentration-dependent effects of (A) rat CgA_{1-64} with intact disulfide bridge ($rCgA_{1-64S-S}$) and (B) human wild-type catestatin (WT-Cts) on isoproterenol (ISO)-stimulation of the left ventricular pressure (LVP) of the rat heart and (C) of bovine catestatin (Cts) on the stroke volume (SV) of the frog heart. Modified from Cerra et al. (Cerra et al., 2008), Angelone et al. (Angelone et al., 2008b) and Mazza et al. (Mazza et al., 2008).

Cts, the CA release-inhibitory peptide: an antihypertensive cardioactive counter-regulator

Recently, cardiac investigators have concentrated on Cts, whose sequence is located close to the C-terminal region of the pro-hormone, corresponding to human $CgA_{352\text{-}372}$ or bovine $CgA_{344\text{-}364}$ (Mahata et al., 1997; Mahata et al., 2000; Mahata et al., 2004).

Cts is a strong non-competitive inhibitor of nicotinic receptor-mediated CA release. It inhibits the calcium-dependent CA release as well as the ACh-induced desensitization of the nicotinic receptor itself (Mahata et al., 1997). Of note, through interaction with histamine receptors, Cts exhibits vasorelaxant and antihypertensive characteristics (Kennedy et al., 1998). This antihypertensive profile is documented by a decrease of its plasma levels in patients with essential hypertension (O'Connor et al., 2002). Accordingly, genetic ablation of the CgA (*Chga*) gene in mice increases blood pressure, while pre-treatment of *Chga*-null mice with Cts prevents blood pressure elevation, indicating a direct role of Cts in preventing hypertension (Krüger et al., 2003). Recently, using the Langendorff-perfused rat heart, we documented the direct myocardial and coronary effects of Cts and its mechanisms of action (Angelone et al., 2008b). Cts dose-dependently increased f_H and coronary pressure and decreased left ventricular (LV) pressure (index of contractile activity), rate pressure product (index of cardiac work) and both positive and negative LV dP/dt (index of maximal rate of left ventricular contraction and relaxation, respectively). The peptide inhibits PLN phosphorylation, thus controlling SR Ca^{2+} uptake; the inotropic and lusitropic actions were abolished by chemical inhibition of β_2-ARs, $G_{i/o}$ protein, NO or cGMP, indicating involvement of β_2-ARs-$G_{i/o}$ protein–NO–cGMP signaling mechanisms. Cts also inhibited ET-1-induced positive inotropism and coronary constriction (Angelone et al., 2008b). From these data, Cts emerges as a novel cardiac modulator, capable of protecting the mammalian heart against excessive sympathochromaffin overactivation, as in the case of hypertensive cardiomyopathy. Using the isolated avascular amphibian (*R. esculenta*) heart, Mazza et al. demonstrated that Cts dose-dependently decreases stroke volume and stroke work, with a threshold concentration of 11 nmol l^{-1}, which is approaching the *in vivo* level of the peptide (Mazza et al., 2008). As in the rat heart, Cts reduces contractility by inhibiting PLN phosphorylation, and its action is abolished by pre-treatment with either NOS (L-NMMA) or cGMP (ODQ) inhibitors or an ET-1 receptor (ET_B) antagonist (BQ788). Of note, Cts non-competitively inhibits the ISO-dependent positive inotropism and the ET-1-induced positive inotropism mediated by ET_A without influencing the ET_B-induced negative-inotropism ET_B involvement is further supported by the evidence that, in the presence of BQ788, Cts failed to inhibit both the ISO- and ET-1-elicited positive inotropic effects, as well as PLN phosphorylation. Taken together, these cardiotropic actions of Cts, particularly the β-adrenergic and ET-1 antagonism, support a role for the peptide as an autocrine–paracrine cardiac modulator. Such influence, we argue, can be particularly important under stress conditions, when the heart becomes a preferential target of both adrenergic and ET-1 stimuli.

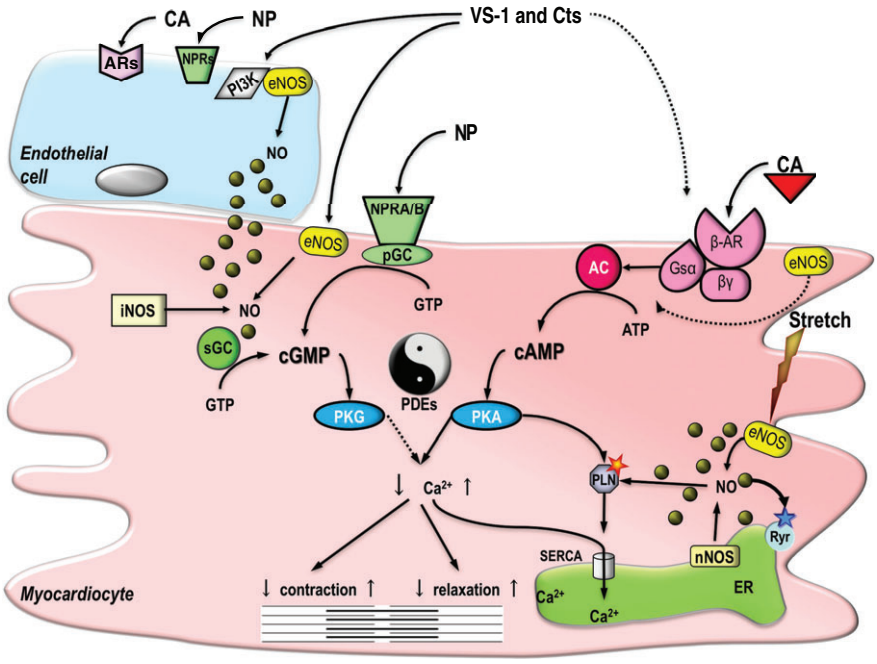

Fig. 6. Diagram of the proposed 'whip–brake' system of the vertebrate heart. It is based on some major connections between CA, NP and CgA-derived peptides, including the integrative function of the NOS–NO system. This simplified scheme incorporates data resulting from different types of cardiac preparations, from whole organ to cultured cells. The convergence of VS-1, Cts, NP and NO on cGMP and the role of this nucleotide in counterbalancing the effects of the CA-dependent cAMP on myocardial performance are shown. The compartmentation of the key enzymes of this network (i.e. pGC, sGC, the three NOS isoforms, PKG, PKA and PDEs), provides the spatial pattern that allows the heart to respond to neuroendocrine stimuli with a fine-tuned regulation of intracellular Ca^{2+} and the consequent modulation of the contraction–relaxation cycle. The yin–yang represents the control operated by PDEs isoenzymes on cGMP and cAMP levels, and thus on the duration and amplitude of their signaling. The orange star on PLN indicates activation of this enzyme by both PKA-dependent phosphorylation and NO-dependent nitrosylation. The stretch-induced autocrine NO production and the NO-dependent blunting of the adrenergic signal are also reported. Note that NO produced by nNOS located on ER may augment contractility *via* NO-dependent nitrosylation of the Ryr (blue star). For simplicity, the effects of PKA- and PKG-dependent phosphorylation of sarcolemmal Ca^{2+} channels are not indicated. A detailed description of intracellular cardiac cascades, references and abbreviations is provided throughout the text. See the List of abbreviations for definitions. Solid arrows indicate stimulation; broken arrows indicate inhibition.

Two cardioactive peptides from the same precursor

Apart from species-specific and tissue-specific post-translational processing of the CgA precursor, the similar cardio-suppressive profile of VS-1 and Cts raises the recurring question regarding the physiological significance of the apparently redundant molecular strategy for which two peptides are processed from one precursor to regulate the same function. While further studies are needed to clarify this issue, it is reasonable to consider that, by acting on overlapping or different sites, VS-1 and Cts may subserve subtly different functions, e.g. summation and synergism or potentiation of the target cell responses to other agonists or distinct spatio-temporal compartmentation (cell- and tissue-specific proteolytic processing and release). Apart from these short-term effects, they can function as transmitters of slow events, including medium- or long-term cardiac remodeling. Similarly, the proteolytic cleavage of proANF precursor gives rise in mammals not only to the major form of circulating ANP (ANP1–28) but also to several biologically active peptides from the 98-amino-acid N-terminus of the prohormone (proANF1–30, long-acting sodium stimulator; proANF31–67, vessel dilator; and proANF79–98, kaliuretic stimulator) (Vesely et al., 1994). At least two of them, i.e. vessel dilator and ANP, show almost overlapping properties, being vasodilatory, diuretic and natriuretic (Vesely, 2006). The examples provided by both CgA and proANF illustrate the striking cardiac potential for multilevel interactions between endocrine precursors and their derived peptides.

CA, NP and CgA: cardiac signaling integration

The conserved existence of CA, NP and CgA in the vertebrate cardiac secretory granules suggests that these hormones, in addition to their individual actions, have additional roles closely linked to specific homeostatic requirements of the heart. Perhaps one of the most important of such roles is represented by the refinement of a flexible ‘whip and brake’ system, as first identified in the antagonistic actions of the SNS and the parasympathetic counterpart by Cannon in his ‘wisdom of the body’ view (Cannon, 1932) and more recently revisited in the concept of counter-regulators in ‘zero steady-state error’ homeostasis (Koeslag, 1999). Indeed, since the formulation of the ‘stress response’ theory (Selye, 1936) and Selye’s recognition of visceral organ dysfunction (Selye, 1956), the heart has been identified as a striking paradigm of a stress-injured organ. Extensive literature has now confirmed the major role of the peripheral limbs of the stress system (the SNS, the increased CA levels and the HPA axis) in maintaining the stress-related cardiac homeostasis, enlightening the relevant clinical implications. We have illustrated here, in an evolutionary and biomedical perspective, the potentials of cardiac CA, NP and CgA to function as an integrated ‘whip–brake’ system, particularly under intense cardio-excitatory stimuli, e.g. CA-induced myocardial stress. Although several links remain to be elucidated, this system helps us to envisage how an increased robustness of the information transfer and neuro-endocrine control of the heart can exert powerful protection of the organ function and survival, thus improving the success of the organism. In Fig. 6, we have depicted the better-documented aspects of this cross-talk. This oversimplified scheme illustrates the recruitment of the two major intracellular messengers, i.e. cGMP and cAMP, and their yin–yang relationship. It also highlights the central role of the NOS–NO system as a terminal effector limb of all three: CA, NP and CgA. As briefly discussed below, the nitrergic system shows the potential for organizing network configurations through connection–integration processes. Based on the available literature, the reader

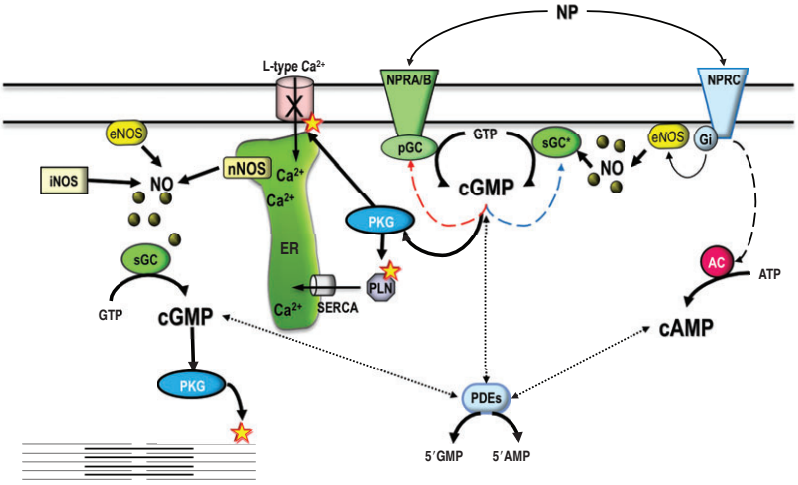

Fig. 7. In cardiac and vascular cells, NP increases cGMP production both directly, *via* activation of NPRA/B-associated pGC, and indirectly, *via* NPRC/G_i-induced stimulation of the eNOS–NO–sGC cascade. This occurs near the membrane in which a caveolin-associated sGC (sGC*) (Venema et al., 2003) subunit is also located. In the cytoplasm and in proximity to the endoplasmic reticulum, cGMP is produced by sGC activated by NO. These subcellular locations allow cGMP to interact downstream with proximal PDE isoforms (e.g. cGMP-activated PDE2 and cGMP-inhibited PDE3), thereby contributing to the cGMP/cAMP balance. Note the potentiation of cGMP signaling sustained by the NPRC-mediated inhibition of cAMP production. At the same time, the high amounts of cGMP generated close to the membrane feed back on pGC and on the membrane-associated sGC*, inhibiting enzyme activity and limiting cGMP production [see Cerra and Pellegrino (Cerra and Pellegrino, 2007) for references]. This feedback may be either homologous (e.g. pGC-generated cGMP *vs* pGC: red broken arrow) or heterologous (e.g. sGC-generated cGMP *vs* pGC: blue broken arrow). PDEs contribute to control cGMP by subtracting the nucleotide from the environment, thus preventing its accumulation. In all these cases, cGMP production, diffusion and signaling are down-regulated. Note that, consistent with its spatial restrictions, cGMP produced close to the membrane mainly acts to decrease intracellular calcium, while cytosolic cGMP decreases myofilament calcium sensitivity (Su et al., 2005). The spatial compartmentation of cGMP- and cAMP-dependent PDEs isoforms is not indicated and their specific role in regulating cyclic nucleotide signaling is oversimplified [for an extensive review, see Bendera and Beavo (Bendera and Beavo, 2006)]. See the List of abbreviations for definitions. Solid arrows indicate stimulation; broken arrows indicate inhibition; dotted arrows indicate both positive and negative effects. The yellow stars indicate phosphorylation.

may incorporate in the proposed scheme additional convergent/divergent pathways, thus increasing the possibilities for interconnections. An example of this is shown in Fig. 7. The diagram illustrates the regulation of the two cGMP-producing systems, i.e. NP-pGC and NO-sGC (soluble guanylate cyclase) and the cGMP-dependent homologous and heterologous negative feedbacks on sGC and pGC. It also illustrates the importance of the spatial restrictions of cGMP downstream cascades in determining the effects of NP and NO on intracellular Ca^{2+} and thus on myocardial contractility (Su et al., 2005; Bender and Beavo, 2006; Cerra and Pellegrino, 2007).

NOS–NO-dependent paracrine-autocrine signaling integration

The intracardiac NOS–NO system appears to be located both at the downstream and at the crossroads of many extrinsic and intrinsic neuro-endocrine, as well as local humoral, pathways [see, for example, eNOS localization close to that of β-adrenoceptors in the caveolae, which attenuates adrenergic stimulation (Barouch et al., 2002)]. As best evidenced in mammals, including humans, NOS isoenzymes (eNOS, nNOS and iNOS), localized in almost every heart tissue, regulate – through their distinct spatial subcellular compartmentation – the production of NO close to its molecular targets (Figs 6 and 7) (Seddon et al., 2007). NO, generated in one cell, can act on one or more processes of the cell itself (autocrine modulation) or on the adjacent cell (paracrine modulation) (Moncada et al., 1991). According to classical NO signaling, defined as the sGC–cGMP-dependent mechanism, NO binds to and activates sGC, leading to a several hundredfold enhancement of cGMP synthesis, with consequent physiological effects (myocardial contractility, relaxation and energetics, smooth muscle relaxation, neurotransmission, etc.). A non-classical, sGC–cGMP-independent mechanism is *S*-nitrosation [also known as *S*-nitrosylation, but see Mitchell et al. for definition (Mitchell et al., 2007)], through which NO converts a cysteine thiol of a target protein to a nitrosothiol (Lancaster and Gaston, 2004; Hess et al., 2005). Like protein phosphorylation, this posttranslational modification is emerging as a fundamental regulation exerted by NO in biological systems, including the heart (Tota et al., 2007b). An example is provided in Fig. 6, in which the effects of NO-dependent nitrosylation on PLN and Ryr are illustrated.

While stretch-induced myocardial stimuli, such as those operating in the Frank–Starling response, appear to involve autocrine NO with consequent inotropic and lusitropic modulation [e.g. PLN phosphorylation and nitrosylation in the eel (Garofalo et al., 2009)], chemical stimuli, such as blood-borne endocrine and humoral agents, appear to exert their cardiotropic effects through the activation of EE–NOS and consequent modulation of the subjacent myocardium, mainly *via* a cGMP-dependent mechanism (Fig. 6). For example, in the eel heart, the decreases of stroke volume (SV) and stroke work (SW) elicited by either β_3-ARs stimulation (Imbrogno et al., 2006) or endoluminal exposure to ANG II (Imbrogno et al., 2003) or VS peptides (Imbrogno et al., 2004) are achieved through this EE–NO–cGMP transduction pathway. This pathway is also involved in the negative inotropic effect of ACh, ET-1 or Cts in the frog heart (Gattuso et al., 1999; Mazza et al., 2008). Therefore, as first proposed for the mammalian heart (Brutsaert, 2003), the EE represents a major sensor–transducer interface for orchestrating the cross-talk between the intraluminal physical/chemical stimuli and the beating myocardium, at the same time contributing through a direct and sustained inotropic action to intracavitary cardiac autoregulation (Sys and Brutsaert, 1995).

Conclusions

The coordinated expression of a large number of different endocrine substances that, together with the extrinsic and intrinsic neurotransmitters, achieve the heart's visceral integration underlines a fascinating aspect of heart plasticity. Namely, the organ capability of detecting, interpreting and responding to short-, medium- and long-term variations in internal and external environments. The integration of information is a fundamental homeostatic task in a complex system, like the continuously beating heart that must not only function in terms of beat-to-beat responses but also adapt to often dramatic ontogenetic and phylogenetic constraints (growth- and hemodynamic load-dependent remodelings, temperature, pH, oxygen and CO_2 changes, osmotic and dehydration challenges, etc.). This implies the existence of information transfer between the elements of the system, as modeled, for example, by the 'bow tie' organizational framework [see Csete and Doyle (Csete and Doyle, 2004) and references therein]. In other words, a new dimension of cardiac biology is emerging in which many inputs ('fan in') converge on a core ('knot') made of a limited number of elements. In the core, the inputs are elaborated and produce a large variety of responses ('fan out'). Thus, the robustness and the flexibility of the biological systems are remarkably increased. Accordingly, although such an increasingly elevated number of cardiac endocrines may appear enigmatic, challenging our present understanding, we can guess that they indeed increase the heart ability to control a range of different activities in its cell and tissue components, augmenting, at the same time, the redundancies (safety factors) and the efficiency of information transfer.

We hope that the whip–brake system of the vertebrate heart proposed in this article will provide a useful platform for a number of questions amenable to signaling-based scientific inquiry, including cardiac plasticity and memory, neurovisceral integration and stress response.

List of abbreviations

ACh	acetylcholine
ANF	atrial natriuretic factor
ANG II	angiotensin II
ANP	atrial natriuretic peptide
AR	adrenoceptor
BNP	B-type natriuretic peptide
CA	catecholamines
CgA	chromogranin A
CNP	C-type natriuretic peptide
Cts	catestatin
DNP	*Dendroaspis* natriuretic peptide
EDRF	endothelium-derived relaxing factor
EE	endocardial endothelium
ER	endoplasmic reticulum
ET-1	endothelin-1
f_H	heart rate
GC	guanylate cyclase
HPA	hypothalamus–pituitary–adrenal
ISO	isoproterenol
LV	left ventricle
NO	nitric oxide
NP	natriuretic peptides
NPR	natriuretic peptide receptor
P13K	phosphatidylinositol 3-kinase
PDEs	phosphodiesterases
pGC	particulate guanylate cyclases
PKA	cAMP-dependent protein kinase
PKG	NO–cGMP–cGMP-activated protein kinase
PLN	phospholamban

P_{O_2}	oxygen partial pressure
PTx	pertussis toxin
RAS	renin–angiotensin system
RNP	renal natriuretic peptide
Ryr	ryanodine receptor
SERCA	sarcoplasmic reticulum Ca^{2+}-ATPase
sGC	soluble guanylate cyclase
SNS	sympathetic nervous system
SR	sarcoplasmic reticulum
VNP	ventricular natriuretic peptide
VS	vasostatins
VS-1	vasostatin 1

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