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# Adenosinergic System in the Mesenteric Vessels

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## 1. Introduction

### 1.1 Adenosinergic pathways in the cardiovascular system

Adenine-based purines, such as adenosine, and adenosine triphosphate (ATP), are ubiquitous signalling molecules that mediate diverse biological actions and physiological processes. Adenosine is an important signalling molecule in the brain, lungs, kidneys, heart, blood vessels and immune systems (Lu et al., 2004), that exerts a potent action on many physiological processes including vasodilation, hormone and neurotransmitter release, platelet aggregation, and lipolysis (Baldwin et al., 2004; Podgorska et al., 2005). Reports of adenosine and adenosine monophosphate (AMP), effects on the heart and blood vessels (Drury & Szent-Gyorgyi, 1929), were the first in a major line of research concerning the physiological actions of purines. Since then, the list of biological processes in which extracellular purines participate has dramatically increased. Insights into the physiological roles of purines came from studies of their biological sources and the stimuli for their release.

Adenosine is composed of an adenine base, consisting of two carbon-nitrogen rings, bound to a ribose sugar group via a beta glycosidic link (Fig. 1); it is considered a nucleoside due to the absence of phosphate groups in its structure. It presents a short half-life due to its rapid conversion into inosine by adenosine deaminase, phosphorylation by adenosine kinase and rapid uptake by adenosine transporters into tissues (Thorn & Jarvis, 1996).

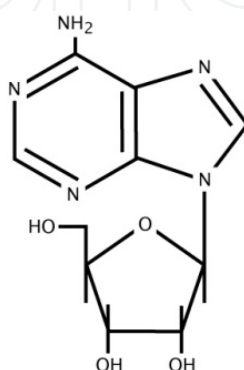


Fig. 1. Adenosine molecule.

Adenosine is a plurisystem mediator/modulator, influencing responses in various cell and tissue types, and *via* numerous receptor and cell signalling pathways. Adenosine can be generated by intracellular and extracellular enzyme pathways depending upon the specific and unique conditions, giving rise to elevated extracellular concentrations. Both equilibrative and concentrative adenosine transport proteins can move adenosine across cellular membranes, influencing extracellular adenosine concentrations (Conlon et al., 2005).

There are several pools of adenosine which arise from different sources. Firstly, there is the existing adenosine being transported in and out of cells *via* transporters. ATP present in the cytosol is dephosphorylated to AMP which can be dephosphorylated further by the action of adenosine kinase to produce adenosine. Alternatively, ATP can be released from the cell by exocytosis, which can then be acted upon by nucleotidases to form adenosine diphosphate (ADP), then AMP and finally adenosine. It can then be transported between the inside of the cell and the interstitial fluid *via* transporters. Another pool of adenosine is generated by neurons. ATP, as a neurotransmitter can be released into the interstitial fluid when carrying a nerve impulse. As before, ATP is acted on by nucleotidases to ADP which is further hydrolysed to AMP and then adenosine (Rang et al., 2007).

Under physiological conditions, adenosine is produced intracellularly (Fig. 2) by AMP dephosphorylation, and extracellularly (Fig. 2) by dephosphorylation of released adenine nucleotides (Brunton et al., 2006; Rang et al., 2007), mainly ATP (Conlon et al., 2005; Meghji et al., 1992).

## 1.2 Adenosine receptors

The intra and extracellular concentration of adenosine is determined, nearby their receptors, by the existence and function of the transporters. Adenosine is a potent modulator of cardiovascular function and when administered systemically, adenosine produces hypotension and bradycardia (Barraco et al., 1987; Evoniuk et al., 1987). These effects are thought to be mediated at adenosine receptors localized centrally (central nervous system) and in the periphery (heart and vasculature), through different receptor subtypes, particularly the adenosine A<sub>1</sub> and A<sub>2A</sub> subtypes (Dhalla et al., 2003; Shryock & Belardinelli, 1997; Spyer & Thomas, 2000; Tabrizchi & Bedi, 2001). In the periphery, A<sub>1</sub> receptors are located primarily in the heart and mediate negative inotropic and chronotropic effects (Shryock & Belardinelli, 1997). Adenosine A<sub>2A</sub> receptors are located primarily in the vasculature and mediate vasodilation (Tabrizchi & Bedi, 2001). In the central nervous system, adenosine A<sub>1</sub> receptors are widely distributed, while adenosine A<sub>2A</sub> receptors are found in limited regions of the brain, most prominently in the striatum (Dunwiddie & Masino, 2001). However, high levels of A<sub>2A</sub> receptors are also found in the cardiovascular regulation regions of the hindbrain, including the nucleus tractus solitarius and the rostral ventral lateral medulla (Thomas et al., 2000). In fact, adenosine A<sub>2A</sub> receptors are thought to play a neuromodulatory role in baroreceptor reflex control (Barraco et al., 1988; Schindler et al., 2005; Thomas et al., 2000).

Adenosine receptors activation may alter vascular tonus in normotensive rats (Cox, 1979; Fresco et al., 2002; Fresco et al., 2004; Fresco et al., 2007), and its modulation differs in hypertensive rats. Thus, it is conceivable that the availability of adenosine may be altered in pathological conditions (Karoon et al., 1995), such as hypertension (Rocha-Pereira et al., 2009).

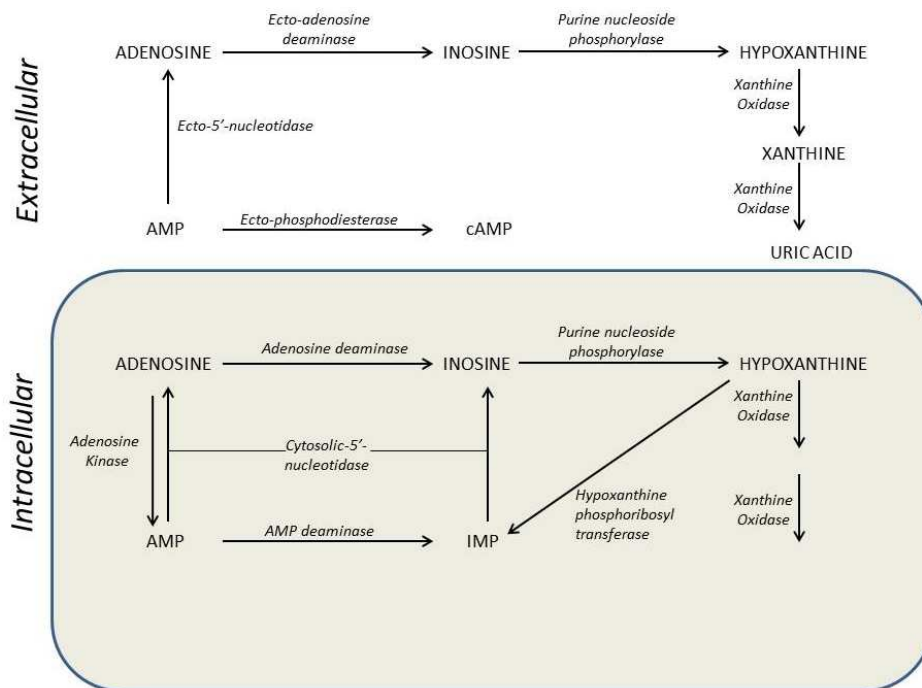


Fig. 2. Metabolism of Adenosine: extracellular. Partial schema of enzyme pathways involved in the regulation of extracellular adenosine concentrations. Cyclic adenosine monophosphate (cAMP) can be transported out of cells upon activation of adenylate cyclase. The actions of an ecto-phosphodiesterase on cAMP results in the formation of AMP. AMP can also be directly released by some cell types. AMP is acted upon by an ecto-5'-nucleotidase to form adenosine; it can then be transported into the cell, or deaminated to inosine by adenosine deaminase. Hypoxanthine is formed after removal of ribose from inosine by the actions of purine nucleoside phosphorylase. Hypoxanthine enters the xanthine oxidase pathway to sequentially form xanthine and uric acid, generating oxyradicals as a byproduct; Metabolism of Adenosine: intracellular. Partial schema of enzyme pathways involved in the regulation of intracellular adenosine concentrations. Adenosine monophosphate (AMP) can be directly deaminated to inosine monophosphate (IMP) by AMP deaminase, or acted upon by an endo-5'-nucleotidase to form adenosine; it can be rephosphorylated to AMP by adenosine kinase, or deaminated to inosine by adenosine deaminase. IMP can also be a source of inosine by the same endo-5'-nucleotidase. Hypoxanthine is formed after removal of ribose from inosine by the actions of purine nucleoside phosphorylase. Hypoxanthine can be salvaged to IMP by hypoxanthinephospho-ribosyltransferase, or by entering the xanthine oxidase pathway to sequentially form xanthine and uric acid, generating oxyradicals as a byproduct. Intracellular adenosine can be transported into and out of the cell by membrane-associated transporter proteins. Being an endogenous purine nucleoside, adenosine is constitutively present in the extracellular spaces at low concentrations. However, its levels increase dramatically in blood and interstitial fluids (extracellular level), in response to cell injury and metabolically-stressful conditions such as tissue damage, hypoxia, ischemia and inflammation. Extracellular adenosine levels have been observed to increase by dephosphorylation of ATP and so a large amount of adenosine is produced from the breakdown of adenine nucleotides by ecto-5'-nucleotidase (Fig. 2) (Cronstein, 1994; Li et al., 2009; Li et al., 2011); and then to be released through the action of specialized nucleoside transporters (Pastor-Anglada et al., 2001).

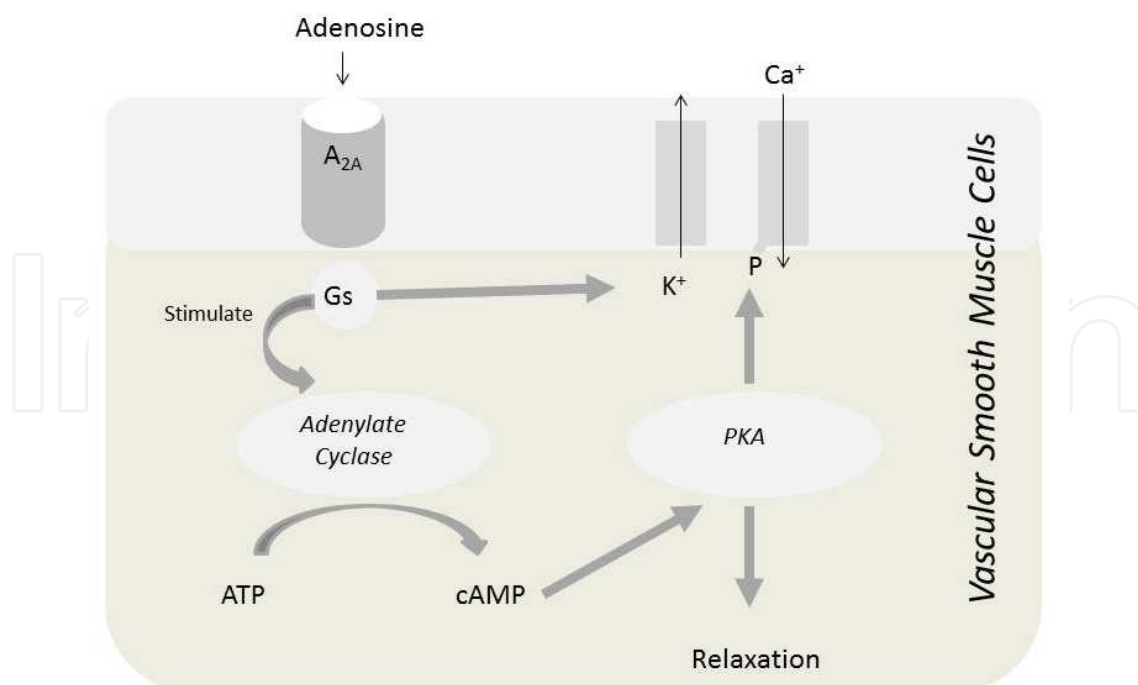


Fig. 3. Adenosine binding to purinergic receptors in smooth muscle tissue. Adenosine can bind to purinergic receptors in different cell types where it can produce diverse physiological actions. One important action is vascular smooth muscle relaxation, which leads to vasodilation. This mechanism is particularly important for matching coronary blood flow to the metabolic needs of the heart. In coronary vascular smooth muscle, adenosine binds to adenosine receptors A<sub>2A</sub>, which are coupled to the G<sub>s</sub>-protein. Activation of this G-protein stimulates adenylate cyclase, increases cAMP and causes protein kinase activation. This stimulates K<sub>ATP</sub> channels, which hyperpolarize the smooth muscle, causing relaxation. Increased cAMP also causes smooth muscle relaxation by inhibiting myosin light chain kinase, which leads to decreased myosin phosphorylation and a decrease in contractile force. There is also evidence that adenosine inhibits Ca<sup>2+</sup> entry into the cell through L-type Ca<sup>2+</sup> channels. Since Ca<sup>2+</sup> regulates smooth muscle contraction, reduced intracellular Ca<sup>2+</sup> causes relaxation. In some types of blood vessels, there is evidence that adenosine produces vasodilation through increases in cGMP, which leads to inhibition of Ca<sup>2+</sup> entry into the cells as well as opening of K<sup>+</sup> channels.

It is well established that adenosine effects occur *via* activation of specific membrane receptors, known as A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub> (Olsson & Pearson, 1990; Ralevic & Burnstock, 1998) that are currently accepted to be coupled to G<sub>i/o</sub>, G<sub>s</sub>, G<sub>s</sub>/G<sub>q</sub> and G<sub>i/o</sub>/G<sub>q</sub> respectively (Fredholm et al., 2001). Adenosine receptors are broadly grouped into two categories: A<sub>1</sub> and A<sub>3</sub> receptors, which couple to inhibitory G proteins, and A<sub>2A</sub> and A<sub>2B</sub> receptors, which couple to stimulatory G proteins. However, adenosine receptors are pleiotropic; they can couple with various G proteins and transduction systems according to their degree of activation and their particular cellular or subcellular location (Cunha, 2005).

### 1.2.1 Adenosine receptors and vasodilation

Adenosine receptors are present in many areas of the organism including the smooth muscle cells of blood vessels - these subtypes of receptors have been found to be distributed

in different blood vessels such as the coronary artery, pulmonary artery, mesenteric artery, renal vasculature and aorta (Olah et al., 1995; Olah & Stiles, 1995).

The importance of the adenosine induced vasodilatation (Fig. 3), is known in the coronary artery of many species including rats. The vasodilatory effect appears to be mediated by  $A_2$  receptors on vascular smooth muscle cells, thus increasing blood flow and oxygenation; also, adenosine released during preconditioning by short periods of ischemia followed by reperfusion can induce cardioprotection to subsequent sustained ischemia (Li et al., 2011). There are two pathways which can result in relaxation. The first pathway is *via* activation of  $A_2$  receptors located on smooth muscle cells, which are linked to  $K_{ATP}$  sensitive channels, and the second is through activation of  $A_2$  receptors located on nitric oxide associated endothelial cells. Alternatively there are blood vessels such as the pulmonary artery in which vascular control is mediated *via* both  $A_1$  and  $A_2$  receptor activation, with vasoconstriction occurring *via* the activation of  $A_1$  receptors and vasodilatation mediated by the activation of  $A_2$  receptors (Tabrizchi & Bedi, 2001).  $A_2$  receptor agonists, including 5'-N-ethylcarboxamide-adenosine, were investigated on porcine coronary artery by King and co-workers (King et al., 1990), and their findings showed that these compounds caused vasodilation. These results support the idea that the activation of adenosine  $A_2$  receptors on smooth muscle results in adenosine-induced relaxation. On the other hand, no evidence has been linked to  $A_3$  receptor activation producing relaxation of blood vessels. Similar findings were obtained from a study by Hiley and co-workers (Hiley et al., 1995), which tested the effects of adenosine analogues on rat mesenteric artery which showed that adenosine analogue, 5'-N-ethylcarboxamide-adenosine acts on adenosine  $A_2$  receptors on the mesenteric bed to produce relaxation. In addition, relaxation mediated by adenosine receptors in the mesenteric bed was sensitive to inhibition by 8-(3-chlorostyryl)caffeine, a selective adenosine  $A_{2A}$  receptor antagonist.

Signalling of the adenosine receptor occurs via a G-protein coupled mechanism, with differences between the subtypes: the  $A_1$  subtype is thought to be coupled to  $G_i$  or  $G_o$  proteins via inhibition of adenylate cyclase or activation of phospholipase C, respectively, and conducting to the opening of  $K^+$  channels and inhibition of  $Ca^{2+}$  channels; the  $A_{2A}$  subtype ( $A_{2AR}$ ) interacts with the G protein  $G_s$  and the  $A_{2B}$  subtype ( $A_{2BR}$ ) interacts with the G proteins  $G_s$  or  $G_q$  to induce adenylate cyclase activity and elevate cAMP levels and consequently, activating calcium channels; the  $A_3$  subtype couples to the  $G_i$  or  $G_q$  proteins through activation of phospholipase C/D or inhibition of adenylate cyclase, respectively (Olah & Stiles, 1995).

It is conceivable that adenosine cardioprotective effect is mediated through the activation of adenosine receptors  $A_1$  and  $A_3$  in cardiomyocytes, and involves protein kinase C and mitochondrial  $K_{ATP}$  channels. However, a recent study has shown that  $A_{2B}$  receptors may also be involved since adenosine  $A_{2B}$  receptor-deficient mice are more susceptible to acute myocardial ischemia and the treatment of normal mice with an agonist of the receptor  $A_{2B}$  significantly attenuated the infarct size after ischemia (Li et al., 2011).

It has been established that adenosine receptor activation occurs via a series of signalling pathways as a result of the binding of adenosine. The affinity of these receptors for adenosine varies; thus their activation depends on the adenosine's concentration. Metabolism and transport across the plasma membrane are the main factors influencing the adenosine level (Podgorska et al., 2005).

In summary, when adenosine binds to these receptors it can cause vascular smooth muscle relaxation leading to vasodilatation of blood vessels. As mentioned above, this occurs *via* a G-protein coupled protein mechanism. On activation of the G-protein, adenylate cyclase is activated causing an increase in cAMP concentration. This then leads to protein kinase A activation which stimulates K<sup>+</sup> channels, hyperpolarizing smooth muscle, causing relaxation (Tawfik et al., 2005).

### 1.3 Nucleoside transporters

Membrane transporters are responsible for the uptake of essential nutrients, modulation of concentrations of physiologically relevant chemicals, and active release of substances such as signaling molecules (Hyde et al., 2001). Transmembrane transport is a critically important physiological process in all cells and, is likely to have evolved early to allow for controlled uptake and release of nonlipophilic compounds. Nucleoside Transporters constitute a family of membrane proteins with different pharmacological and kinetic properties (Fredholm, 2003), recently identified and characterized in humans. These transport proteins were initially purified from human blood red cells for more than two decades ago, and the lack of abundance of nucleoside transporters proteins in the membranes of mammalian cells, has hampered the analysis of the relationship between its structure and function (Endres et al., 2009; Molina-Arcas et al., 2008; Molina-Arcas et al., 2009).

As previously discussed, there are several ways in which adenosine can be produced and made available for adenosine receptors. One of such, being the transport of adenosine across the plasma membrane, through nucleoside transporters, which determine the intra and extracellular levels of nucleosides, including adenosine (Baldwin et al., 2004; Lu et al., 2004). Generally, nucleoside transporters facilitate the movement of nucleosides and nucleobases across cell membranes but their distribution is not homogeneous among tissues, and their expression can be regulated by various physiological and pathophysiological conditions (Baldwin et al., 2004; Lu et al., 2004; Molina-Arcas et al., 2008). Over the past two decades important advances in the understanding of nucleoside transporters functioning have been achieved. One of nucleoside transporters functions is to salvage extracellular nucleosides for intracellular synthesis of nucleotides; besides, they also control the extracellular concentration of adenosine in the vicinity of its cell surface receptors and regulate processes such as neurotransmission and cardiovascular activity (Anderson et al., 1999; Cass et al., 1999). Other function of nucleoside transporters is vital for the synthesis of nucleic acids in cells that lack *de novo* purine synthesis: carrier-mediated transport of this nucleoside plays an important role in modulating cell function, because the efficiency of the transport processes determines adenosine availability to its receptors or to metabolizing enzymes. Therefore, nucleoside transporters may be key elements as therapeutic targets in the cardiovascular disorders as they are, for example, in anticancer and antiviral therapy where nucleoside analogues are successfully used (Huber-Ruano & Pastor-Anglada, 2009; Lu et al., 2004; Molina-Arcas et al., 2005; Yao et al., 2002).

To date it is accepted that there are two types of transporters (Fig. 6) (Baldwin et al., 2004; Podgorska et al., 2005):

- Equilibrative Nucleoside Transporters (ENT) - equilibrative bidirectional transport processes driven by chemical gradients by facilitated diffusion. ENT are present in

most, possibly all, cell types (Cass et al., 1998). They might mediate adenosine transporter in both directions, depending on the concentration gradient of adenosine across the plasma membrane. Until the present day, there are four subtypes described: ENT1, ENT2, ENT3 and ENT4 (Baldwin et al., 2004; Molina-Arcas et al., 2009; Podgorska et al., 2005).

- Concentrative Transporters (CNT) – active inwardly directed concentrative processes, driven by the  $\text{Na}^+$  electrochemical gradient:  $\text{Na}^+$ -dependent. CNT are expressed in a tissue-specific fashion (Cass et al., 1998). Three subtypes were described: CNT1, CNT2 and CNT3 (Hyde et al., 2001; Kong et al., 2004; Molina-Arcas et al., 2009).

Identification and molecular cloning of the ENT and CNT families from mammals and protozoan parasites have provided detailed information about the structure, function, regulation, tissue and cellular localization (Baldwin et al., 2004; Molina-Arcas et al., 2008). Comparing these different types of transporters, CNT and ENT, some differences become evident. Whereas the CNT transport processes are present primarily in specialized epithelia, the ENT transport processes are found in most mammalian cell types (Cass et al., 1998).

Both types of transporters are tightly regulated, both by endocrine and growth factors and by substrate availability. They transport endogenous substrates such as adenosine, thymidine, cytidine, guanosine, uridine, inosine, and hypoxanthine (Lu et al., 2004). They are both involved in the transport of adenosine, but ENT have higher affinity for adenosine than CNT (Molina-Arcas et al., 2009), a reason why the present study focused exclusively on ENT.

ENT play an important role in the provision of nucleosides, derived from the diet or produced by tissues such as the liver, for salvage pathways of nucleotide synthesis in those cells deficient in *de novo* biosynthetic pathways. The latter include erythrocytes, leukocytes, bone marrow cells and some cells in the brain. The co-existence in many cell types of both ENT1 and ENT2, which exhibit similar nucleoside specificities, may reflect the importance of the ENT2 substrate hypoxanthine as a source of purines for salvage. Similarly, this ability to transport hypoxanthine and the higher apparent affinity of ENT2 for inosine have been suggested to reflect a role in the efflux or uptake of these adenosine metabolites during muscle exercise and recovery respectively (Baldwin et al., 2004; Endres et al., 2009). Several polymorphisms have been described in ENT proteins that could affect nucleoside homeostasis, adenosine signalling events or nucleoside-derived drug cytotoxicity or pharmacokinetics (Kong et al., 2004; Molina-Arcas et al., 2009). Although the transport of adenosine involves a simple carrier system, it is a complex process.

### 1.3.1 Equilibrative nucleoside transporters isoforms

The first example of the ENT family was characterized in human tissues at the molecular level only 10 years ago. Since that time, the identification of homologous proteins by functional cloning and genome analysis has revealed that the family is widely distributed in eukaryotes. The SLC29 family of integral membrane proteins, is part of a larger group of equilibrative and concentrative nucleoside and nucleobase transporters found in many eukaryotes. ENT are a unique family of proteins with no apparent sequence homology to other types of transporters, which enable facilitated diffusion of nucleosides, such as adenosine, and nucleoside analogues across cell membranes (Hyde et al., 2001). Studies performed over the past thirty years have revealed that most mammalian cells exhibit low-



affinity, ENT processes, now known to be mediated by members of the SLC29 family. Some mammalian ENT have been well characterized at the molecular and pharmacological levels (Crawford et al., 1998), and currently, four isoforms are known: ENT1–4 (Hyde et al., 2001).

Human (h) and rat (r) ENT1 and ENT2 (456–457 amino acid residues) transport both purine and pyrimidine nucleosides, including ADO. They also differ in their sensitivity to vasodilator drugs (hENT1 > hENT2 > rENT1 > rENT2) and by the ability of hENT2 and rENT2 to transport nucleobases as well as nucleosides (Hyde et al., 2001).

ENT family members are predicted to possess 11 transmembrane helices, with a cytoplasmic N-terminus and an extracellular C-terminus experimentally confirmed for ENT1 (Baldwin et al., 2004). The number of molecules present of each ENT subtype depends on both the cell and the tissue type. The intra and extracellular concentration of adenosine is determined, nearby their receptors, by the existence and function of the transporters, and the four isoforms although structurally similar, show differences in their ability to regulate adenosine concentrations, which may be due to slight modifications in configuration (Baldwin et al., 2004).

Whilst the name of the family reflects the properties of its prototypical member ENT1, some family members can also transport nucleobases and some are proton-dependent, concentrative transporters. Therefore, the transporters play key roles in nucleoside and nucleobase uptake for salvage pathways of nucleotide synthesis, and are also responsible for the cellular uptake of nucleoside analogues. In addition, by regulating the concentration of adenosine available to cell surface receptors, they influence many physiological processes ranging from cardiovascular activity to neurotransmission (Baldwin et al., 2004). ENT are targets, for example, for coronary vasodilator drugs, are responsible for the cellular uptake of nucleoside analogues used in the treatment of cancers and viral diseases (Elwi et al., 2006; Young et al., 2008) and they can also act as routes for uptake of cytotoxic drugs in humans and protozoa (Hyde et al., 2001).

The best-characterized members of the family, ENT1 and ENT2, are cell surface proteins that possess similar broad substrate specificities for purine and pyrimidine nucleosides regulating, eventually, the access of adenosine to its receptors. ENT1 plays a primary role mediating adenosine transport while ENT2, in addition, efficiently transport nucleobases (Baldwin et al., 2004). More recently, the ENT3 and ENT4 isoforms have been shown to be also genuine nucleoside transporters, they are both pH sensitive, and optimally active under acidic conditions. ENT3 has a similar broad permeant selectivity for nucleosides and nucleobases and appears to function in intracellular membranes, including lysosomes. ENT4 is uniquely selective for adenosine, but yet present a low affinity to this nucleoside, and it may also transport a variety of organic cations (Baldwin et al., 2004; Kong et al., 2004).

All four isoforms are widely distributed in mammalian tissues, although their relative abundance varies. In polarised cells ENT1 and ENT2 are found in the basolateral membrane and, in tandem with CNT of the SLC28 family, may play a role in transepithelial nucleoside transport. ENT2 is known to be particularly abundant in skeletal muscle while the ENT3 isoform seems to be widely distributed and the most abundant ENT in the heart. Nevertheless, since ENT3 is a lysosomal transporter functioning in intracellular membranes, is unlikely to contribute to a direct regulation of interstitial adenosine concentrations in tissues. Finally, in what concerns the ENT4, it presents low sequence identity to the other

members of the family (due to differences in its structure), is highly selective for adenosine and is also widely distributed. For instance, ENT4 is present in vascular endothelial cells and contributes to regulate the extracellular concentration of adenosine in these structures but only at acidic pH (Baldwin et al., 2004; Barnes et al., 2006).

In summary, all four members of the family share an ability to transport adenosine, but differ in their abilities to transport other nucleosides and nucleobases.

The human gene encoding the human ENT1 (hENT1) protein has been localized to region p21.1-21.2 on chromosome 6 (Baldwin et al., 2004). hENT1 protein consists of 456-residue protein and its sequence displays about 78% identity to the 457-residue rat homologue (rENT1) and 79% identical to the 460-residue mouse protein (mENT1.1) homologues. Splice variants of hENT1 have not been reported, but a 458-residue variant of the mouse homologue (mENT1.2), generated by alternative splicing at the end of exon 7, is widely distributed (Abdulla & Coe, 2007).

The two forms of mENT1 protein appear to be functionally identical, although mENT1.2 lacks the potential casein kinase II phosphorylation site. Both rENT1 and hENT1 proteins display broad substrate specificity for pyrimidine and purine nucleosides with  $K_m$  values ranging from 50 mM (adenosine) to 680 mM (cytidine), but are unable to transport the pyrimidine base uracil (Yao et al., 1997). hENT1 and mENT1, which are sensitive to nitrobenzylthioinosine (NBMPR), are also inhibited by the coronary vasodilators dipyridamole, dilazep, and draflazine. In contrast, rENT1 although presenting sensitivity to NBMPR is essentially insensitive to inhibition by the coronary vasodilators dipyridamole and dilazep (Baldwin et al., 2004; Podgorska et al., 2005; Ward et al., 2000; Yao et al., 1997).

The messenger ribonucleic acid (mRNA) for hENT1 is widely distributed in different tissues, including erythrocytes, liver, heart, spleen, kidney, lung, intestine, and brain (Endres et al., 2009; Griffith & Jarvis, 1996; Lum et al., 2000; Pennycooke et al., 2001). mENT1.2 protein was shown to be commonly co-expressed with mENT1.1 (460 aminoacids) and the highest level was found in the liver, heart and testis. Moreover, studies at both the mRNA and protein levels have revealed that ENT1 is almost ubiquitously distributed in human and rodent tissues, although its abundance varies between tissues (Baldwin et al., 2005).

Human ENT2 (hENT2) protein, responsible for the *ei* type nucleoside transport, is encoded by a gene localized at position 13q on chromosome 11. hENT2 consists of 456 aminoacids and their sequence displays 88% identity to mouse (mENT2) and rat (rENT2) homologues. In humans, besides the 456-aminoacid ENT2 protein, exists at least, two shorter forms of ENT2, generated from mRNA splice variants. The 326 aminoacid protein, termed hHNP36, lacks the first three transmembrane domains and is inactive as a nucleoside transporter. Inactive is also the second splice variant, a 301-aminoacid protein named hENT2A that lacks the C-terminal domain (Crawford et al., 1998).

The ENT2 protein accepts a broad range of substrates, including purine and pyrimidine nucleosides and nucleobases. It has been postulated that hENT2 plays a role in the efflux and reuptake of inosine and hypoxanthine generated from adenosine during and after strenuous physical exercise. ENT2 (both rat and human), is much less susceptible to inhibition by NBMPR and the coronary vasodilators dipyridamole and draflazinethan ENT1 (Baldwin et al., 2004; Crawford et al., 1998; Podgorska et al., 2005; Ward et al., 2000; Yao et al., 1997, 2002).

The mRNA for ENT2 was reported to be present in several tissues including heart, kidney, brain, placenta, thymus, pancreas, intestine and prostate, but the highest expression level was found in skeletal muscle (Crawford et al., 1998; Lum et al., 2000; Pennycooke et al., 2001).

The gene encoding the human ENT3 (hENT3) protein is located at position q22.1 on chromosome 10. hENT3 is a 475-residue protein displaying 73% identity to the mouse homologue (mENT3) (Baldwin et al., 2004, 2005; Kong et al., 2004). ENT3 has a characteristic, long (51 aminoacids), hydrophilic N-terminal region preceding the first transmembrane (TM1) domain. The N-terminal region of ENT3 consists of two di-leucine motifs characteristic for endosomal, lysosomal targeting motifs. This architectural design distinguishes the ENT3 protein from other members of the equilibrative transporters family. Indeed, it was demonstrated that hENT3 protein is predominantly localized intracellularly and that mutation of the dileucine motif to alanine triggers the relocation of ENT3 protein to the cell surface (Baldwin et al., 2004, 2005).

In comparison with ENT1, the ENT3 protein is much less susceptible to inhibition by NBMPR and coronary vasodilatory drugs (dipyridamole and dilazep). hENT3 demonstrates a broad selectivity for nucleosides, but does not transport hypoxanthine. Moreover, the hENT3 protein facilitates transport of several adenosine analogues like cordycepin (3'-deoxyadenosine) (Baldwin et al., 2004; Podgorska et al., 2005). hENT3 and hENT4, which are mainly located in the intracellular organelles, are not prominent nucleoside transporters like hENT1 and hENT2 (Endo et al., 2007).

The mRNA for ENT3 has been detected in a variety of mouse and human tissues, including brain, kidney, colon, testis, liver, spleen, placenta (highest level), and in a number of neoplastic tissues (Baldwin et al., 2004, 2005; Hyde et al., 2001).

The gene encoding the human ENT4 (hENT4) protein is located on chromosome 7, at position p22.1. Interestingly, the hENT4 is more closely related to the products of the *Drosophila melanogaster* gene CG11010 (28% identity) and the *Anopheles gambiae* gene agCG56160 (30% identity), than to hENT1 (18% identity), indicating an ancient divergence from the other members of the SLC29 family (Acimovic & Coe, 2002). hENT4 is a 530-residue protein 86% identical in sequence to its 528-residue mouse homologue (mENT4) (Baldwin et al., 2004). The substrate specificity of hENT4 has not yet been established in detail, but among the ENT proteins, hENT4 has the lowest affinity for adenosine (Kong et al., 2004). The mRNA for hENT4 was detected in several human tissues. However, recent characterisation of the complementary deoxyribonucleic acids (cDNAs) encoding h/mENT4 has confirmed that these proteins are indeed nucleoside transporters, capable of low-affinity adenosine transport. Analysis of multiple tissue RNA arrays indicates that hENT4 is likely to be ubiquitously expressed in human tissues (Baldwin et al., 2005; Podgorska et al., 2005).

### 1.3.2 Equilibrative nucleoside transporters in the cardiovascular system

There are currently no reports implicating ENT - SLC29 transporters family, in the pathogenesis of human disease (Baldwin et al., 2004). Still, as mentioned above, adenosine transporters contribute to the intra and extracellular concentration of adenosine, modulating its concentration in the vicinity of its receptors (Li et al., 2011; Tawfik et al., 2005). It is therefore conceivable that the availability of adenosine may be altered in pathological states.

Adenosine exerts vasodilatory and cardioprotective effects, and also reduces the proliferation of vascular smooth muscle cells, inhibits platelet aggregation and attenuates the inflammatory response. Apart from adenosine receptors and ecto-5'-nucleotidase, transporter proteins can regulate adenosine function by modulating extracellular levels of adenosine. The extracellular adenosine is rapidly taken up into cells by nucleoside transporters and is, subsequently, metabolized to inosine by adenosine deaminase and phosphorylated to AMP by adenosine kinase. Nucleoside transporters are supposed to play an integral part in adenosine functions by "fine-tuning" local levels of adenosine in the vicinity of adenosine receptors (Li et al., 2011).

Recent studies have proposed the occurrence of a greater degree of adenosine release from cells that are metabolically stressed. In other words, cells with a high oxygen demand such as the vascular smooth muscle cells in the hypertensive state (Conlon et al., 2005; Tabrizchi & Bedi, 2001). Several studies have been conducted in order to further understand the role of ENT in cardiovascular diseases (Chaudary et al., 2004; Li et al., 2011; Reyes et al., 2010; Rose et al., 2010). Adenosine seems to be a cardioprotective metabolite. Hypoxia and ischemia lead to a large increase in extracellular adenosine, which is released by cardiomyocytes. Extracellular adenosine activates G-protein coupled adenosine receptors linked to various signalling pathways, which initiate compensatory responses. Intracellular and extracellular levels of adenosine fluctuate, considerably, depending on the metabolic state of the heart, the flux of adenosine (down its concentration gradient), across the cardiomyocyte cell membrane, is facilitated by the ENT. These transporters are highly expressed in the cardiovascular system but very little is known about their role in cardiomyocyte physiology (Baldwin et al., 2004; Chaudary et al., 2004; Reyes et al., 2010).

As previously mentioned, ENT are bidirectional, allowing adenosine to be released from cells (to act as an autocrine/paracrine hormone), or transported into the cell (to terminate receptor activation, or restore adenosine metabolite pools). Thus, cardiomyocyte adenosine physiology is dependent on the adenosine receptor profile, and on the presence and activity of the ENT. In the past years, ENT have been shown to be important in modulating the effects of adenosine in human epithelial cells. Moreover, a correlation was found between ENT1 and A<sub>1</sub> adenosine receptor distribution in the brain, suggesting potential interactions and/or feedback between receptors and transporters. Nevertheless, there is an extensive literature on adenosine and adenosine receptor physiology in the cardiovascular system, whereas very little is known about ENT (Baldwin et al., 2004; Chaudary et al., 2004).

ENT inhibitors, by virtue of their effect on extracellular adenosine concentrations, can also modulate a variety of physiological processes, potentially leading to therapeutic benefits. For example, by inhibiting nucleoside uptake into endothelial and other cells the coronary vasodilator drafazine substantially increases and prolongs the cardiovascular effects of adenosine. The latter exerts beneficial, cardioprotective effects in the ischaemic/reperfused myocardium mediated, at least in part, *via* activation of A<sub>1</sub> and possibly also A<sub>3</sub> receptors, probably involving the protein kinase C and mitochondrial K<sub>ATP</sub> channels. Transport inhibitors have also potential value in the context of ischaemic neuronal injury: pre-ischaemic administration of the pro-drug NBMPR phosphate has been shown to increase brain adenosine levels and reduce ischaemia-induced loss of hippocampal neurons in the rat. In a clinical setting, pharmacological inhibition of ENT, using drugs such as

dipyridamole, dilazep and draflazine, is used to promote cardiovascular health. However, despite the clinical relevance of ENT as drug targets, very little is known about them (Baldwin et al., 2004; Tabrizchi & Bedi, 2001; Takahashi et al., 2010).

Recent studies have challenged the role of ENT in purine nucleoside-dependent physiology of the cardiovascular system. Rose and co-workers (2010), investigated whether the ENT1-null mouse heart was cardioprotected in response to ischaemia. In that study, the authors observed that ENT1-null mouse hearts showed significantly less myocardial infarction compared with wild-type littermates, demonstrating that ENT1 activity may contribute to cardiac injury. A posterior study (Reyes et al., 2010), confirmed that isolated wild-type adult mouse cardiomyocytes express predominantly ENT1, which is primarily responsible for purine nucleoside uptake in these cells. However, ENT1-null cardiomyocytes exhibit severely impaired nucleoside transport and lack ENT1 transcript and protein expression. Adenosine receptor expression profiles and expression levels of ENT2, ENT3, and ENT4 were similar in cardiomyocytes isolated from ENT1-null adult mice compared with cardiomyocytes isolated from wild-type littermates. Moreover, small interfering RNA knockdown of ENT1 in the cardiomyocyte cell line, mimics findings in ENT1-null cardiomyocytes. Taken together, the data from the study conducted by Rose and co-workers (2010), demonstrated that the absence of ENT1 plays an essential role in cardioprotection, most likely due to its effects in modulating purine nucleoside-dependent signalling and that the ENT1-null mouse is a powerful model system for the study of the role of ENT in the physiology of the cardiomyocyte.

Other authors, determined that adenosine and inosine accumulate extracellularly during hypoxia/ischaemia and that both may act as neuroprotectors (Takahashi et al., 2010). In the spinal cord, there was pharmacological evidence for an extracellular adenosine levels increase during hypoxia, but no direct measurements of purine release have been done; furthermore, the efflux pathways and origin of extracellular purines are still not defined. Therefore, to characterize hypoxia-evoked purine accumulation, Takahashi and co-workers (2010), examined the effect of acute hypoxia on the extracellular levels of adenosine and inosine in isolated spinal cords from rats, and these authors found that both inhibitors of adenosine deaminase or ENT, abolished the hypoxia-evoked increase in inosine but not adenosine: extracellular level of inosine was about 10-fold higher than that of adenosine. These data suggest that hypoxia releases adenosine itself from intracellular sources, on the other hand, inosine formed intracellularly may be released through ENT (Takahashi et al., 2010).

Gestational diabetes has been associated with increased L-arginine transport and nitric oxide (NO) synthesis as well as a reduced adenosine transport in human umbilical vein endothelial cells. Adenosine increases endothelial L-arginine/NO pathway via A<sub>2</sub> adenosine receptors in human umbilical vein endothelial cells, in normal pregnancies (Vasquez et al., 2004; Vega et al., 2009) compared to the reduction in adenosine transport observed in veins of women with gestational diabetes. Additionally, an association between L-arginine transport and NO synthesis was also found. In fact, Vázquez and co-workers (2004), demonstrated that in gestational diabetes, stimulation of L-arginine transport and NO synthesis occurs with a reduction in adenosine transport in human umbilical vein endothelial cells.

The effect of gestational diabetes on the L-arginine/NO pathway may result from an increased extracellular adenosine level, due to low adenosine uptake as a consequence of a reduced *hENT1* mRNA expression. Accumulation of extracellular adenosine could activate  $A_{2A}$  adenosine receptors, which leads to an increased expression of cationic amino acid transporter-1 (*hCAT-1*) mRNA, and of endothelial nitric oxide synthase (*eNOS*), mRNA or protein expression, an increased L-arginine transport activity, as well as, of the NO synthesis. The effect of gestational diabetes on adenosine and L-arginine transport involves activation of protein kinase C, and p42/44 MAPK pathways and increased the NO levels. Thus, the authors hypothesized the establishment of a functional link between adenosine transport and the L-arginine/NO pathway, governing the normal function of human fetal endothelium from gestational diabetic pregnancies (Vasquez et al., 2004; Vega et al., 2009).

These results also highlight the physiological effects of purinoceptors, particularly of adenosine receptors, in the umbilical vein endothelium, in pathologies, where alterations of blood flow from the mother to the fetus (via the umbilical vein may occur), altering the normal supply of nutrients to the developing fetus, such as in intrauterine growth restriction, fetal hypoxia or gestational diabetes. Finally, these findings also demonstrate that gestational diabetes induces alterations in the phenotype of human fetal endothelium (Vasquez et al., 2004).

It has been demonstrated that insulin inhibited elevated ENT1 expression in human umbilical arterial smooth muscle cells from pregnancies in diabetic subjects (Aguayo et al., 2001). However this is probably due to the activation of adenylate cyclase rather than the effect of insulin on glucose metabolism. The effects of oral anti-diabetic agents on nucleoside transporters are rarely reported: Li and co-workers (2011), studied the effects of different oral anti-diabetic agents such as metformin, sulfonyureas, meglitinides and thiazolidinediones on nucleoside transporters; among them, only the thiazolidinedione troglitazone showed inhibitory effects on nucleoside transporters, but unfortunately it was withdrawn because of hepatic toxicity.

To our knowledge, until the present date, only one study has been carried out to investigate the relationship between hypertension and nucleoside transporters. The binding of a ENT1 probe [ $^3\text{H}$ ]NBMPR in membranes prepared from platelets, as well as renal, pulmonary, cardiac and brain tissues of Spontaneously Hypertensive Rats (SHR), was compared to those of age matched Wistar-Kyoto (WKY) controls (Williams et al., 1990). The number of [ $^3\text{H}$ ]NBMPR binding sites were higher in the kidneys of SHR but lower in platelets, whereas no difference was found in the heart, lung or brain. Age-dependent decreases were also observed in the heart and platelets of SHR and WKY. The results indicated that the expression of ENT1 changed with age as well as with the pathogenesis of hypertension. Li and co-workers (2011), compared the expressions of nucleoside transporters in basilar arteries in SHR and WKY rats and they found that ENT1 and ENT2 were unaffected by hypertension. Interestingly, the mRNA expression of CNT2 was higher than that seen in WKY; nevertheless, whether the upregulation of CNT2 is a primary or secondary event in the development of hypertension is questionable. It has been speculated that the increase in the activities of ENT1 and CNT2 may reduce the availability of adenosine to its receptors, thereby weakening the vascular functions of adenosine. It may explain why patients with diabetes and hypertension suffer greater morbidity from ischemia and atherosclerosis (Li et al., 2011).

## 2. Mesenteric vessels

The branches of the abdominal aorta are divided into parietal and visceral parts. The visceral arteries are in turn divided into paired and unpaired branches. The mesenteric artery is an elasto-muscular resistance vessel. In adult rats, for example WKY, the mesenteric artery branches from the abdominal aorta and is composed of five to seven concentric layers of smooth muscle cells, separated by three to four medial laminae. The medium is separated from the endothelial cells of the intima by the continuous internal elastic lamina and from the adventitia, which contains a few fibroblasts and nerve terminals, by the external elastic lamina (McGuire et al., 1993; Sullivan et al., 2002).

Three major unpaired branches exist: the celiac trunk, the superior mesenteric artery and the inferior mesenteric artery. Each has several major branches supplying the abdominal organs: the superior mesenteric artery, supplies the pancreas, small intestine and the colon, whereas the inferior mesenteric artery supplies the descending colon and rectum (Fig. 4).

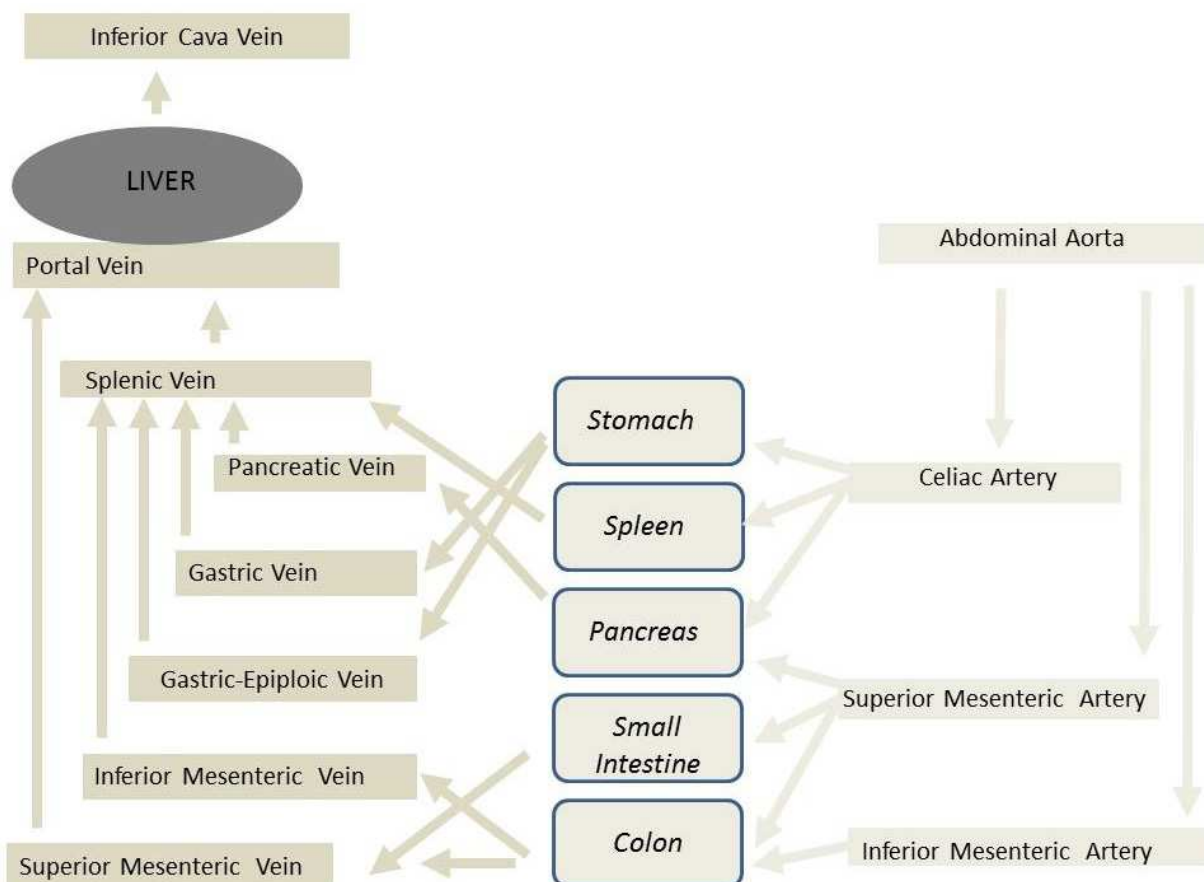


Fig. 4. Schematic representation of splanchnic circulation. Under normal resting conditions in humans, total hepatic blood flow is 1200 to 1400 mL/min (~100 mL/min/100g), which represents about 25% of cardiac output. Blood flow to the four lobes of the liver is derived from two major sources, the portal vein and the hepatic artery. The hepatic artery is a branch of the celiac axis and accounts for 25 to 30 percent of total hepatic blood flow and 45 to 50 percent of the oxygen supply. The portal vein is a valveless afferent nutrient vessel of the liver that carries blood from the entire capillary system of the stomach, spleen, pancreas, and intestine (McGuire et al., 1993).

The mesenteric circulation plays an important role in maintenance of systemic blood pressure, and regulation of tissue blood flow. Actually, the entire splanchnic circulation can receive up to 60% of cardiac output and contains about one third of the total blood volume. Mesenteric arteries and veins have significant resistance and capacitance functions in the systemic circulation, respectively. In comparison to the associated veins, the mesenteric artery has a high resting basal tone mediated in part by a thicker layer of vascular smooth muscle (Kreulen, 2003). Constriction of the mesenteric artery is thought to increase total peripheral resistance in the systemic circulation greatly. In contrast, the mesenteric vein, contains fewer layers of vascular smooth muscle cells and are more compliant vessels. The function of these low pressure vessels is to store significant quantities of blood that can be utilized to maintain the central venous pool of blood and cardiac output. As such, the degree of vascular tone in mesenteric vasculature plays a major role in the regulation of systemic blood pressure and overall body hemodynamics (Ross & Pawlina, 2006).

### 2.1 Vascular tonus regulation and physiology in mesenteric vessels

The tone of the mesenteric artery and resistance blood vessels are mainly regulated by sympathetic adrenergic nerves through the release of neurotransmitter noradrenaline. It is also controlled by nonadrenergic noncholinergic nerves, and possibly by parasympathetic cholinergic nerves. Noradrenaline and adrenergic cotransmitters including neuropeptide Y, and ATP, act as a vasoconstrictor neurotransmitter for sympathetic nerves. While, dopamine, calcitonin gene-related peptide and acetylcholine act as a vasodilator neurotransmitter for adrenergic, nonadrenergic noncholinergic and cholinergic nerves, respectively. In the mesenteric circulation, these nerves containing various neurotransmitters and cotransmitters interact and modulate each other *via* feedback autoregulatory mechanisms and neuromodulation of various vasoactive substance to regulate vascular resistance (Takenaga & Kawasaki, 1999). In fact, net vascular tone in the mesenteric vasculature is under the influence of several key factors. These factors include locally acting and circulating hormones, intrinsic myogenic properties of the vessel, as well as neurotransmitters released from perivascular post-ganglionic sympathetic neurons. In general, the arteries and veins of the splanchnic circulation are richly innervated with sympathetic nerves that act to constrict these vessels. Maximal activation of the sympathetic constrictor nerves can produce an 80% reduction in blood flow to the splanchnic region (Morhrman & Heller, 2006).

*In vivo*, sympathetic neurogenic influence of vascular tone is mediated by three neurotransmitters: neuropeptide Y, noradrenaline, and ATP make up the sympathetic triad of neurotransmitters. Perivascular neurons store the sympathetic neurotransmitters in synaptic vesicles and release these neurotransmitters from varicosities to act on postjunctional receptors on the vascular smooth muscle cells. The arrangement of the sympathetic neurons differs between arteries and veins. The nerve plexus for the mesenteric artery consists of a bundle of axons arranged in a mesh-like network with nerve fibres equally likely to run parallel or perpendicular to the longitudinal axis of the vessel. In contrast, in mesenteric vein the nerve plexus consists of single axons with a circumferential nerve fibre arrangement about the vessel. In both cases, the sympathetic neurotransmitters released cause depolarization of the nearby vascular smooth muscle cells. As a whole, activation the sympathetic postjunctional receptors mediates contraction of the vascular smooth muscle cells and, therefore, constriction of the artery or vein (Park et al., 2007).



*In vitro*, electrical field stimulation studies have found that upon stimulation of mesenteric perivascular nerves a measurable amount of noradrenaline is released (Bobalova & Mutafova-Yambolieva, 2001). Once released, noradrenaline can act on a variety of receptors on the vascular smooth muscle cells. Previous *in vitro* studies have found contractile responses to be mediated by activation of the  $\alpha_1$  adrenoceptor in mesenteric arteries and both  $\alpha_1$  and  $\alpha_2$  adrenoceptors in the mesenteric vein (Perez-Rivera et al., 2007). These adrenoceptors are G-protein linked receptors that are coupled to an intracellular increase of inositol 1,4,5-triphosphate (IP3). Contraction of the smooth muscle is mediated by IP3 acting on sarcoplasmic reticulum receptors to release intracellular  $\text{Ca}^{2+}$  stores. Additional  $\text{Ca}^{2+}$  is taken up into the vascular smooth muscle cells following depolarization via L-type voltage-gated calcium channels (Lee et al., 2001).

Like noradrenaline, a measurable amount of ATP is released from perivascular sympathetic neurons when activated by electrical field stimulation (Bobalova & Mutafova-Yambolieva, 2001). *In vivo*, ATP is thought to mediate neurogenic contractions of vascular smooth muscle by acting on various purinergic receptors present on the smooth muscle cells. The two subtypes of purinergic receptors are the P2X and P2Y receptors. The P2X receptors are ATP-gated ion channels that cause an influx of  $\text{Ca}^{2+}$  into the smooth muscle cells from the extracellular environment (Donoso et al., 2004). Though there are several P2X receptor isoforms, there is evidence that vascular smooth muscle cells primarily express the P2X1 receptors (Wang et al., 2002). P2X receptors are thought to be responsible for the excitatory junction potentials (rapid and short depolarization of vascular smooth muscle) present in mesenteric artery (Kreulen, 2003). In contrast, excitatory junction potentials are not present in mesenteric vein. This is largely thought to be the result of selective expression of only the P2Y receptor subtype in mesenteric vein (Mutafova-Yambolieva et al., 2000). The P2Y receptors mediate slower contractile responses than the P2X receptors, and are G-protein linked receptors that have similar intracellular effects as the  $\alpha$ -adrenoceptors. The isoforms of the P2Y receptors that are thought to be expressed in vascular smooth muscle cells are the P2Y2 and the P2Y4 receptors (Galligan et al., 2001).

Noradrenaline and ATP contract the mesenteric artery and the mesenteric vein through the activation of adrenergic and purinergic vascular smooth muscle receptors respectively. The use of selective adrenoceptor agonists and antagonists suggests that the  $\alpha_1$  adrenoceptor is the primary adrenoceptor mediating responses to noradrenaline in these vessels. In addition, the data collected suggests that the P2X and/or the P2Y1 receptors contract the mesenteric artery, but do not mediate substantial contractile responses in rat mesenteric vein. Therefore, this data suggests that other purinergic receptors, such as the P2Y2 and the P2Y4 receptor subtypes, mediate vasoconstriction in these vessels in response to ATP. The Sympathetic Nervous System, is an important modulator of net vascular tone in mesenteric arteries and veins, and that sympathetic modulation of these vessels is an important regulator of the resistance function of mesenteric artery and the capacitance function of mesenteric vein.

Splanchnic veins and venules account for most of the active capacitance responses in the circulation and are richly innervated by the Sympathetic Nervous System. In fact, it has been estimated that innervation to the non hepatic splanchnic organs accounts for half of the total noradrenaline released in the entire body. Therefore, the recent observations in Angiotensin II salt hypertension of neurogenically mediated increases, in whole body venous tone,

would best be explained by increased Sympathetic Nervous System activity to the splanchnic circulation. The splanchnic vascular resistance rises in proportion to the blood pressure, and the transvascular escape rate of plasma proteins is increased. Vascular resistance increases in the hepatosplanchnic circulation before any other bed in humans with borderline hypertension. Therefore, increased sympathetic activity to the splanchnic circulation may represent a common stage in the development of hypertension (King et al., 2007).

The various animal models of hypertension show variable results, but in general support the concept that vascular resistance changes in the splanchnic organs are similar in direction and magnitude to pressure changes. These resistance changes appear to result from increased responsiveness of the arterioles to a variety of constrictor influences, and they may result from either structural or functional changes. Hypertension appears to alter splanchnic arteriolar permeability *via* a pressure-dependent mechanism. These vessels may also undergo degenerative histological changes. In addition to the resistive and exchange alterations, the capacitance function of splanchnic veins is reduced, probably via a structural change (Nyhof et al., 1983).

Also, chronic hypertension is associated with resistance artery remodelling and mechanical alterations. A study by Briones and co-workers (2003), evaluated the role of elastin in vascular remodelling of mesenteric artery from SHR. When compared with WKY, the mesenteric artery of SHR showed: smaller lumen, decreased distensibility at low pressures, a leftward shift of the stress-strain relationship, redistribution of elastin within the internal elastic lamina leading to smaller fenestrae but no change in fenestrae number or elastin amount. Elastase incubation fragmented the structure of internal elastic lamina in a concentration-dependent fashion, abolished all the structural and mechanical differences between strains, and decreased distensibility at low pressures. Mesenteric artery remodelling and increased stiffness are accompanied by elastin restructuring within the internal elastic lamina and elastin degradation reverses structural and mechanical alterations of SHR mesenteric artery. Differences in elastin organisation are, therefore, a central element in small artery remodelling in hypertension (Briones et al., 2003).

The rat superior mesenteric vein, which drains blood from the intestine, or the splenic vein, which drains from the spleen, is a capacitance vein. The blood flow in the superior mesenteric vein is the primary source of irrigation of the rat liver and this vein plays an important role in maintaining bile flow, bile acid excretion, and bilirubin conjugation and in preventing the precipitation of bile (possibly preventing hepatolithiasis) (Adachi et al., 1991).

The splanchnic venous bed is the largest vascular bed in terms of capacitance because 50% of the intestinal blood volume is in the venules and small mesenteric veins (Dunbar et al., 2000). Also, many animal studies have shown that the splanchnic bed is very responsive to baroreceptor and sympathetic stimulation (Haase & Shoukas, 1991, 1992; Shoukas & Bohlen, 1990), pointing to this vascular bed as a primary source of blood volume changes. These studies have demonstrated that sympathetic stimulation of splanchnic veins and venules will cause them to constrict, leading to significant volume shifts out of this vascular bed. Consequently, changes in splanchnic venous capacitance can have large effects on venous filling pressure. Capacitance changes can occur through changes in both vessel compliance and unstressed vascular volume. One way to experimentally assess these changes is to

examine changes in the pressure-diameter relationships of individual vessels, particularly in the splanchnic regions of the body. The mesenteric veins are also critical in modulating cardiac filling through venoconstriction.

The mesenteric circulation is regulated by multiple mechanisms and there is sufficient amount of aspects described in the literature that support the suspicion that local metabolic factors are especially important in the control of intestinal vasculature. Of these, adenosine, which is a mesenteric vasodilator, may be the messenger of the intestinal tissue to signal appropriate responses of the intestinal vessels. The evidence supporting the candidacy of this nucleoside as a local regulator of mesenteric circulation may be summarized, as follows: adenosine is present in the tissue of the gut in measurable quantities; exogenous adenosine is a powerful dilator of mesenteric resistance vessels; blockade of adenosine receptors in the mesenteric circulation interferes significantly with three autoregulatory phenomena, i.e., postprandial hyperaemia, pressure-flow autoregulation, and reactive hyperaemia (Jacobson & Pawlik, 1992).

### 3. Adenosinergic system and hypertension

Some lines of investigation have already used both these models to study the role of adenosine in hypertension. For example, in 1987, Jackson performed an interesting assay, where the author compared the *in vivo* role of adenosine, as a modulator of noradrenergic neurotransmission, in the SHR and WKY. In the *in situ* blood-perfused rat mesentery, vascular responses to sympathetic periarterial nerve stimulation, and to exogenous noradrenaline, were enhanced in SHR compared with WKY. In both SHR and WKY, vascular responses to periarterial nerve stimulation were more sensitive to inhibition by adenosine, than were responses to noradrenaline. At matched base-line vascular responses, compared with WKY, SHR were less sensitive to the inhibitory effects of adenosine on vascular responses to periarterial nerve stimulation, but SHR and WKY were equally sensitive with respect to adenosine-induced inhibition of responses to noradrenaline. Antagonism of adenosine receptors with 1,3-dipropyl-8-p-sulphophenylxanthine, shifted the dose-response curve to exogenous adenosine six-fold to the right, yet did not influence vascular responses to periarterial nerve stimulation or noradrenaline in either SHR or WKY. Furthermore, periarterial nerve stimulation did not alter either arterial or mesenteric venous plasma levels of adenosine in SHR or WKY, and plasma levels of adenosine in both strains were always lower than the calculated threshold level required to attenuate neurotransmission. According to these findings, the author concluded that *in vivo* exogenous adenosine interferes with noradrenergic neurotransmission in both SHR and WKY; SHR are less sensitive to the inhibitory effects of exogenous adenosine on noradrenergic neurotransmission than are WKY; endogenous adenosine does not play a role in modulating neurotransmission in either strain under the conditions of this study; and enhanced noradrenergic neurotransmission in the SHR is not due to defective modulation of neurotransmission by adenosine (Jackson, 1987).

Other studies have found differences in blood vessels when comparing SHR and WKY (Cox, 1979; Gisbert et al., 2002; Leal et al., 2008; Lee, 1987; Rocha-Pereira et al., 2009). Findings from the study by Gisbert and co-workers (2002), showed that the population of constitutively active  $\alpha_{1D}$ -adrenoceptors is significantly increased in aorta and mesenteric

artery from adult SHR when compared to WKY - these results verify that the vessels in question, from hypertensive animals, have an increased population of constitutively active receptors as well as an increased functionality of the  $\alpha_{1D}$ -subtype, with respect to normotensive animals. Other studies (Villalobos-Molina & Ibarra, 1999; Villalobos-Molina et al., 1999; Xu et al., 1998), highlighted the importance of the  $\alpha_{1D}$ -adrenoceptor in the pathology of hypertension, suggesting that, it appears first in the vasculature, followed by a rise in blood pressure.  $\alpha_{1D}$ -adrenoceptors can be found on smooth muscle cells and the activation of these receptors by noradrenaline (released by sympathetic postganglionic terminals), conduce mainly to vasoconstriction. These can lead to an increase in blood pressure playing, therefore, a role in the development of hypertension observed in SHR animals. The opposite effect to the contraction of smooth muscle can occur as a result of adenosine acting on  $A_2$  adenosine receptors, thus causing smooth muscle relaxation. The levels of extracellular adenosine, which can act on those receptors to produce this effect, can be altered by ENT. These transporters play a role in determining the levels of adenosine available extracellularly and hence the extent of vasodilation which can occur (Rang et al., 2007).

On the other hand, previous studies have shown that the vasodilatory response to adenosine and its analogues is weakened in hypertension and in other pathological conditions affecting blood vessels (Lockette et al., 1986; Luscher et al., 1987). Lüscher and co-workers (1987) investigated the effects of antihypertensive therapy on hypertensive rats and their findings demonstrated a prevention or reversal of decreased endothelium-dependent relaxations in response to agonists, suggesting that antihypertensive treatment normalizes endothelium-dependent relaxations. It was, therefore, proposed that antihypertensive treatment may be important in preventing cardiovascular complications in hypertensive individuals. A possible explanation for the attenuated vasodilatory response to adenosine in hypertension can be linked to the levels of adenosine receptors and their functionality in hypertensive models. Vasodilation of blood vessels occurs via the activation of adenosine receptors by adenosine and so an alteration in these receptors can result in a modified vasodilatory response (Rocha-Pereira et al., 2009). This hypothesis can be associated with adenosine transporters, which are partly responsible for making adenosine available to adenosine receptors. It is, therefore, legitimate to hypothesize that an increase or decrease in transporter population could indirectly alter the physiology of the adenosinergic system and, contributing indirectly to the contractility of blood vessels and conducting to an elevated blood pressure (correspondent to the pathologic situation of hypertension).

#### **4. Nucleoside transporters as therapeutic tools – Future perspectives**

Nucleoside derived drugs or nucleobase analogues are being developed and investigated for a number of different applications, in order to pursue a better and more efficient way of treating several diseases. The studies conducted in several other conditions might shed some light in this challenging pathway, as promising results have already been published, for example in cancer and infection by Human Immunodeficiency Virus (Cano-Soldado et al., 2008; Damaraju et al., 2003; Endo et al. 2007; Hyde et al., 2001; Kong et al., 2004; Molina-Arcas et al., 2005, 2009; Ritzel et al., 2001; Yao et al., 2002), chronic pain and inflammation (Eltzschig et al., 2005; Li et al., 2009, 2011; Reyes et al., 2010).

The amount of research being done in these past years is indicative of the interest and potential that the area of ENT have in many scientific fields, especially in what concerns its role in a pharmacological perspective. Hopefully, in the future, as the knowledge increases and the mechanisms involving these transporters are better understood, the application of ENT will have an impact, particularly, in cardiovascular diseases, such as hypertension.

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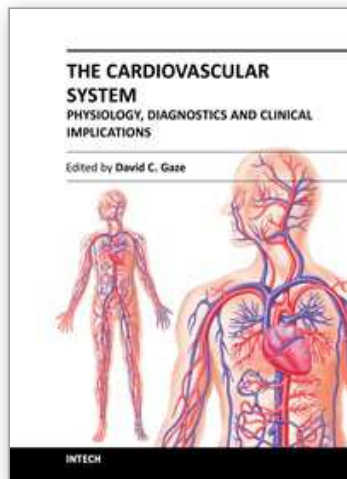
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## **The Cardiovascular System - Physiology, Diagnostics and Clinical Implications**

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The cardiovascular system includes the heart located centrally in the thorax and the vessels of the body which carry blood. The cardiovascular (or circulatory) system supplies oxygen from inspired air, via the lungs to the tissues around the body. It is also responsible for the removal of the waste product, carbon dioxide via air expired from the lungs. The cardiovascular system also transports nutrients such as electrolytes, amino acids, enzymes, hormones which are integral to cellular respiration, metabolism and immunity. This book is not meant to be an all encompassing text on cardiovascular physiology and pathology rather a selection of chapters from experts in the field who describe recent advances in basic and clinical sciences. As such, the text is divided into three main sections: Cardiovascular Physiology, Cardiovascular Diagnostics and lastly, Clinical Impact of Cardiovascular Physiology and Pathophysiology.

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