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Adrenaline and Noradrenaline: Partners and Actors in the Same Play

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1. Introduction

1.1 An overview on the role of catecholamines: Basic physiology and pharmacology

Tremendous advances in the knowledge of catecholamines occurred in the last century, namely the discovery of their chemical structure, the notion of adrenoceptors by Ahlquist or the characterization of (at least in part) their transduction pathways. General concepts related to catecholamines, namely their synthesis, storage, reuptake, release, action termination, the adrenergic receptors, and their transduction pathways will be approached in this chapter. Additionally, the levels of catecholamines and metabolites found in human plasma and urine will be briefly addressed. Special focus will be given to adrenaline (ADR) and noradrenaline (NA) since they are the main actors of the human adaptation to the environment. ADR and NA are responsible for the cross talk between the sympathetic nervous system (and central nervous system) and adrenal medulla.

2. Historic introduction and background

In 1856, Vulpian applied a solution of ferric chloride to slices of adrenal glands and noticed that the medulla stained green while the cortex did not. He also observed that the same reaction occurred in samples of venous blood leaving the adrenal, but not in the arterial blood entering the gland. To account for these observations, Vulpian assumed that the medulla synthesized a substance that was released into the circulation (Vulpian, 1856). In 1895, Oliver and Schäfer demonstrated the first pharmacological action of catecholamines when they showed that the administration of extracts of adrenal gland led to the rise of arterial blood pressure (Oliver and Schäfer, 1895).

In 1897, Abel and Crawford obtained a compound that they called “epinephrin” (Abel and Crawford, 1897). The mono-benzoyl derivative isolated was however less active than the crude extracts, so the quest for the bioactive compound continued. In 1901, Takamine, an industrial chemist, isolated the active principle, then named “adrenalin” (Takamine, 1902) (Figure 1). Takamine’s notation was adopted by the Europeans but not by North American researchers, thus resulting in the different nomenclature of both sides of the Atlantic Ocean.

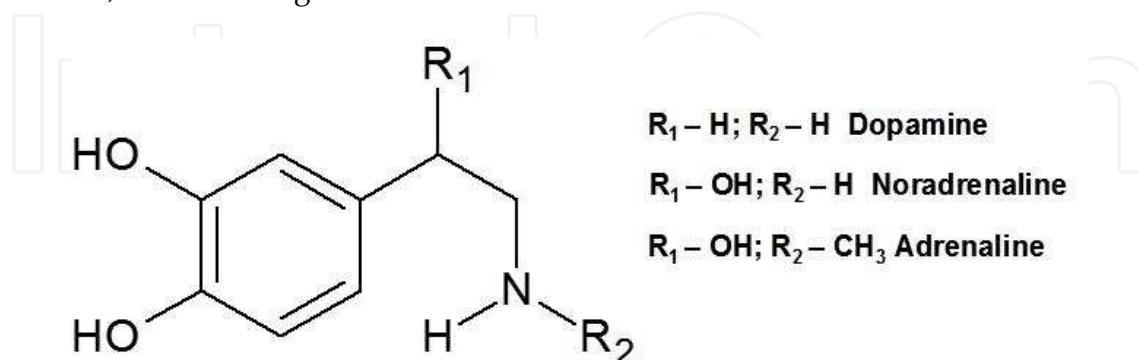


Fig. 1. Chemical structures of biogenic catecholamines.

Catecholamines soon became the centre of one of the most enduring and passionate scientific discussions, with tremendous breakthroughs that crossed the twentieth century. When reporting to catecholamines, under the general heading are NA (also known as norepinephrine), the principal transmitter of sympathetic postganglionic fibres and certain tracts of the central nervous system; dopamine, a known transmitter of the mammalian nigrostriatal, mesocortical, and mesolimbic neuronal pathways; and ADR (also known as epinephrine), the major hormone of the adrenal medulla. Collectively, these three amines are called biogenic catecholamines (Westfall and Westfall, 2006) and are involved in the regulation of motor coordination, learning and memory, sleep-wake cycle regulation, as well as in endocrine and visceral functions (Wevers et al., 1999).

3. Structure and main location of catecholamine-releasing sites

NA, ADR, and dopamine are known as catecholamines, as they contain a catechol moiety, an amine side-chain and they are all derived from the amino acid tyrosine (Westfall and Westfall, 2006, Baynes and Dominiczak, 2007, Rang et al., 2007) (Figure 1). Catecholamines share with 5-hydroxytryptamine (5-HT; serotonin) the amino side chain and altogether are known as biogenic amines. Similar compounds, like isoproterenol (previously isoprenaline), a synthetic derivative of NA, are catecholamines but not biogenic amines (Rang et al., 2007).

Although many before postulated it, von Euler was the first to show that NA was the main neurotransmitter in the sympathetic nervous system (von Euler, 1946). Sympathetic nerves arise from the spinal cord and run to the ganglia situated close to it; from those ganglia the postganglionic noradrenergic nerves run to the target tissues. Stimulation of the sympathetic nerves is inseparable from the “fight or flight” response, which results in the secretion of corticosteroids by the adrenal cortex and the release of ADR and NA by the adrenal medulla and sympathetic nerves (Wang et al., 1999, Baynes and Dominiczak, 2007). Together with ADR and corticosteroids, NA helps to coordinate different body part responses for

adaptation to a stressful situation. The main function of NA is to orchestrate the response of the body to stress culminating in increased heart rate and blood pressure, and enhanced energy mobilization and neural reflexes (Wang et al., 1999, Westfall and Westfall, 2006).

NA is a neurotransmitter found in the peripheral and central nervous system, where it regulates a numerous assortment of physiological processes that include mood, arousal, learning and memory, blood flow, and metabolism (Axelrod and Kopin, 1969, Xu et al., 2000, Nolte, 2009). As a central nervous system neurotransmitter, NA is synthesized primarily in the brainstem *nuclei locus ceruleus* and *subceruleus*, which project rostrally to virtually every region of the midbrain and forebrain, dorsoventrally to the cerebellum, and caudally to lumbar segments of the spinal cord (Wang et al., 1999, Westfall and Westfall, 2006, Nolte, 2009). Additionally, the A1, C1, A2, C2, C3, A4, A5, and A7 noradrenergic cell groups also provide projections into the brain. Noradrenergic projections are identified in virtually every region of the midbrain, forebrain and cerebellum (Nolte, 2009). In the peripheral nervous system, NA is synthesized and released from sympathetic neurons connected to a wide variety of endocrine organs and other tissues (Axelrod and Kopin, 1969, Wang et al., 1999, Nolte, 2009). This extensive projection pattern throughout the neuroaxis defines NA as a general regulator of neurotransmission. NA is directly involved in mood stabilization, sleep regulation, aggression and in the general degree of alertness and arousal. NA is also involved in the central control over the endocrine and autonomic nervous systems (Wang et al., 1999, Westfall and Westfall, 2006, Nolte, 2009).

Although generally treated only as an endocrine hormone, ADR also acts as a neurotransmitter released in central and peripheral adrenergic neurons (Loewi, 1936, Jarrott, 1970, Fuller, 1982). Indeed, Loewi demonstrated the presence of an "acceleranzstoff" released from sympathetic neurons that innervated the heart of frogs (Loewi, 1921), later found to be ADR (Loewi, 1936, Azuma et al., 1965, Norberg and McIsaac, 1967). The presence of ADR in the mammalian central nervous system was recognized both in biochemical and neuroanatomical studies (Lew et al., 1977, Pendleton et al., 1978, Moore and Bloom, 1979, Armstrong et al., 1982, Goodchild et al., 1984). The existence and function of only ADR-synthesizing neurons are not without controversy (Mefford, 1987). The confirmation of specific neurons in the central nervous system that contain ADR occurred after the development of sensitive enzymatic assays and immunocytochemical staining techniques for phenylethanolamine N-methyltransferase (PNMT), the enzyme responsible for the synthesis of ADR (Axelrod, 1962, Lew et al., 1977, Pendleton et al., 1978). Subsequently, neuroanatomical studies using antibodies against PNMT showed the presence of ADR-synthesizing neurons in the C1, C2, and C3 cell groups of the *medulla oblongata* and the *nucleus tractus solitarii* in multiple species (Goodchild et al., 1984, Carlton et al., 1987, Carlton et al., 1991), including humans (Burke et al., 1986, Halliday et al., 1988, Kitahama et al., 1988). Many other studies have shown the existence of adrenergic neurons in the medullar reticular formation that make restricted connections to a few pontine and diencephalic *nuclei*, eventually coursing as far rostrally as the paraventricular nucleus of the dorsal midline thalamus (Armstrong et al., 1982, Chamba and Renaud, 1983, Goodchild et al., 1984, Ross et al., 1984b, Stolk et al., 1984, Bloom, 2006). It is recognized nowadays that ADR synthesizing neurons are involved in cardiovascular homeostasis in both physiological and pathophysiological conditions (Moore and Bloom, 1979, Goodchild et al., 1984, Reis et al., 1984, Ross et al., 1984a, Johansson et al., 1997).

In adults, ADR accounts for approximately 80% of adrenal medulla catecholamine content, with NA making up most of the remaining (von Euler, 1972). Although the adrenal gland and neurons are the most prominent sites of PNMT expression in the adult mammal, this enzyme has also been detected in the retina (Hadjiconstantinou et al., 1983, Foster et al., 1985, Park et al., 1986), spleen (Pendleton et al., 1978), lungs (Pendleton et al., 1978, Kennedy et al., 1990), and heart (Axelrod, 1962, Pendleton et al., 1978, Culman et al., 1987, Torda et al., 1987, Kennedy and Ziegler, 1991). ADR triggers coordinated metabolic and physiological processes in response to stress (Westfall and Westfall, 2006). ADR is more active than NA in the heart and lungs, it causes redirection of blood from the skin to skeletal muscle and it has important stimulatory effects in the glycogen metabolism of the liver (Westfall and Westfall, 2006, Baynes and Dominiczak, 2007, Rang et al., 2007).

Dopamine (3,4-dihydroxyphenylethylamine) is the immediate metabolic precursor of NA and ADR (Figure 2). Although dopamine originally was regarded only as a precursor of

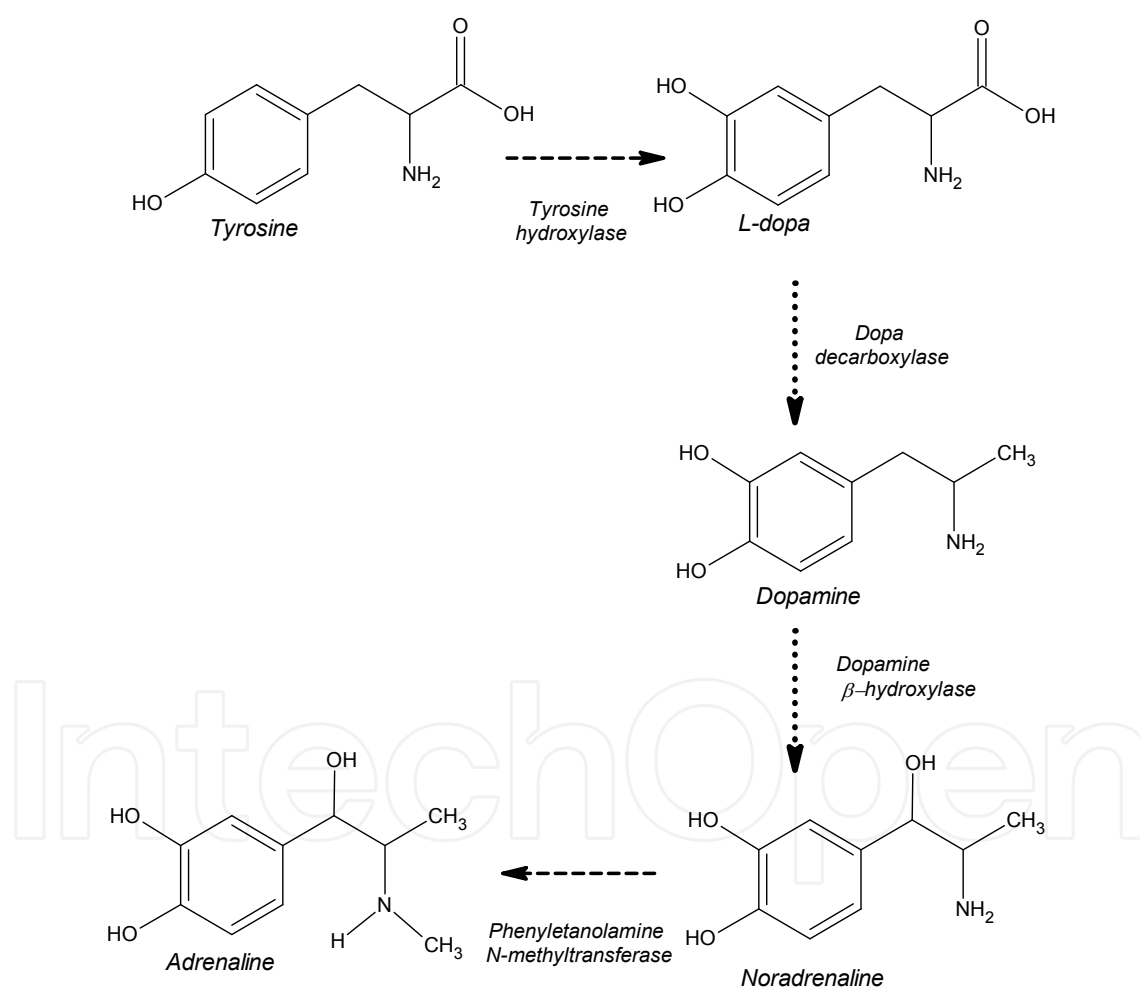


Fig. 2. The synthesis of catecholamines. Tyrosine hydroxylase hydroxylates tyrosine and forms L-3,4-dihydroxyphenylalanine (L-dopa). L-dopa is decarboxylated to form dopamine through the catalysis of dopa decarboxylase. Dopamine β-hydroxylase β-hydroxylates dopamine to yield noradrenaline. Noradrenaline is N-methylated by phenylethanolamine N-methyltransferase to form adrenaline in adrenergic neurons and other adrenaline producing cells.

NA, studies on distinct central nervous system regions revealed that the distribution of dopamine and NA neurons is markedly different (Bloom, 2006). More than half of the central nervous system catecholamine content is dopamine and extremely large amounts are found in the basal ganglia (especially the *caudate nucleus*), the *nucleus accumbens*, the olfactory tubercle, the central nucleus of the amygdala, the median eminence, and restricted fields of the frontal cortex (Bloom, 2006). Of the wide variety of connections, the greatest attention has been given to the long projections between the major dopamine-containing nuclei located at the substantia nigra and ventral tegmentum and their targets in the striatum. In addition, two important central dopaminergic systems are functionally located outside the blood-brain barrier: in the chemoceptor trigger zone and in the anterior pituitary. In the periphery, dopamine is synthesized in epithelial cells of the proximal tubules and it is thought to exert local diuretic and natriuretic effects in the kidneys (Esler et al., 1990, Bloom, 2006). Likewise, dopamine receptors are found in the proximal gastrointestinal tract where their activation delays gastric emptying. Dopaminergic receptors are also present in renal, mesenteric, coronary and intracerebral arteries (Bloom, 2006). At the cellular level, the actions of dopamine depend on the expression of the different receptor subtypes and the contingent actions of other transmitters to the same target neurons (Bloom, 2006). This catecholamine will not be deeply addressed in the present work, except in specific circumstances where it can be of relevance to the theme.

4. Synthesis of catecholamines

The mechanisms of catecholamine synthesis, storage, release, and binding have been mainly studied in sympathetically innervated organs and in the adrenal medulla (Westfall and Westfall, 2006).

The steps that occur in the synthesis of dopamine, NA, and ADR are shown in Figure 2. In the cytoplasm, tyrosine is sequentially 3-hydroxylated by tyrosine hydroxylase to form L-3,4-dihydroxyphenylalanine, which is then decarboxylated to form dopamine by L-3,4-dihydroxyphenylalanine decarboxylase (also known as aromatic L-amino acid decarboxylase) (Axelrod, 1962). Dopamine β -hydroxylase β -hydroxylates dopamine to yield NA, which is N-methylated by PNMT to form ADR, in adrenergic neurons and other ADR producing cells (Rang et al., 2007). The enzymes involved in this synthesis have been identified, cloned, and characterized (Nagatsu, 1991).

In adrenergic neurons, the enzymes that participate in NA formation are synthesized in the cell bodies of neurons and are then transported along the axon to the terminals. In the course of NA synthesis, both the hydroxylation of tyrosine to L-3,4-dihydroxyphenylalanine and the decarboxylation of L-3,4-dihydroxyphenylalanine to dopamine take place in the cytoplasm. About half of the dopamine formed in the cytoplasm is then actively transported into the dopamine β -hydroxylase-containing storage vesicles, where it is converted to NA. The remainder escapes active transport to the vesicles and is deaminated to 3,4-dihydroxyphenylacetic acid and subsequently O-methylated to homovanillic acid (HVA) (Westfall and Westfall, 2006). In ADR-producing cells, the NA formed in the vesicles leaves them and it is methylated in the cytoplasm by PNMT to yield ADR. ADR then re-enters the

vesicles, where it is stored and concentrated for subsequent release (Masson et al., 1999, Westfall and Westfall, 2006).

The hydroxylation of tyrosine by tyrosine hydroxylase is generally regarded as the rate-limiting step in the biosynthesis of catecholamines (Zigmond et al., 1989). This enzyme is activated after stimulation of sympathetic nerves or adrenal medulla and is tightly regulated. In fact, tyrosine hydroxylase is substrate for protein kinase A (PKA), protein kinase C (PKC) and calcium/calmodulin-dependent protein kinase II (CaMKII). The kinase-catalysed phosphorylation results in the increase of tyrosine hydroxylase activity (Zigmond et al., 1989, Daubner et al., 1992). This is an important acute mechanism to increase the synthesis of catecholamines in response to elevated nerve stimulation. Likewise, tyrosine hydroxylase activity is subject to a feedback inhibition by catechol compounds, which allosterically modulate the activity of the enzyme (Kumer and Vrana, 1996). On the other hand, the expression of tyrosine hydroxylase can be increased at multiple levels, including transcription, ribonucleic acid (RNA) processing, regulation of RNA stability, translation, and enzyme stability (Kumer and Vrana, 1996).

Tyrosine hydroxylase deficiency has been reported in humans and is characterized by generalized rigidity, hypokinesia, among other symptoms. Low cerebrospinal fluid levels of NA and dopamine metabolites, like HVA and 3-methoxy-4-hydroxy-phenylethylene glycol are observed in humans with tyrosine hydroxylase deficiency (Wevers et al., 1999, Carson and Robertson, 2002). The tyrosine hydroxylase knockout is unviable in mice as they die in the embryonic stage, presumably because catecholamine loss results in altered cardiac function (Zhou et al., 1995). Interestingly, residual levels of dopamine are present in these embryonic mice, suggesting that tyrosinase may be an alternate way to form catecholamines. However, the amount of tyrosinase-derived catecholamines is clearly not sufficient for the survival of the animals (Carson and Robertson, 2002).

Dopamine β -hydroxylase deficiency in humans is characterized by orthostatic hypotension, ptosis of the eyelids, retrograde ejaculation, and elevated plasma levels of dopamine (Westfall and Westfall, 2006). In the case of dopamine β -hydroxylase-deficient mice, there is about 90% embryonic mortality (Carson and Robertson, 2002).

At rest, the basal cardiovascular function in PNMT knockout mice showed little change. In fact, ADR was found indispensable for normal blood pressure and cardiac filling responses to stress but it was not required in tachycardia during stress or in normal cardiovascular function at rest (Bao et al., 2007, Sun et al., 2008). Furthermore, glucocorticoids levels are very important in the expression of PMNT and thus in the rate of ADR synthesis. Glucocorticoids are very relevant in the size of the stores of ADR available for release in the brain (Moore and Phillipson, 1975b, a), adrenal medulla (Kelner and Pollard, 1985, Stachowiak et al., 1988, Wan and Livett, 1989, Ross et al., 1990, Betito et al., 1992, Wong et al., 1992), heart (Kennedy and Ziegler, 1991, Krizanova et al., 2001, Kvetnansky et al., 2004) or lungs (Kennedy et al., 1993). The activities of both tyrosine hydroxylase and dopamine β -hydroxylase are also increased in the adrenal medulla under the influence of glucocorticoids (Carroll et al., 1991). Thus, any stress that persists sufficiently to evoke an enhanced secretion of corticotrophin mobilizes the appropriate hormones of both the adrenal cortex (predominantly cortisol in humans) and adrenal medulla.

5. Intracellular storage of catecholamines

Neurotransmission depends mainly on the regulated release of chemical transmitter molecules. That release requires the packing of these substances into the specialized secretory vesicles of neurons and neuroendocrine cells. The neuronal content of catecholamines is confined to vesicles, while in adrenal medulla, catecholamines are stored in chromaffin granules. These vesicles contain extremely high concentrations of catecholamines (approximately 21% dry weight), ascorbic acid, and adenosine-5'-triphosphate (ATP), as well as chromogranins, dopamine β -hydroxylase, and peptides, including enkephalin and neuropeptide Y. Two types of storage vesicles are actually found in sympathetic nerve terminals: large dense-core vesicles equivalent to the chromaffin granules and small dense-core vesicles containing NA, ATP, and membrane-bound dopamine β -hydroxylase (Bloom, 2006). The enhanced activity of the sympathetic nervous system is accompanied by increase of both dopamine β -hydroxylase and chromogranins in circulation, thus supporting the argument that the process of release, following the adrenergic nerve stimulation, involves exocytosis (Westfall and Westfall, 2006).

The storage process decreases the intraneuronal metabolism of the transmitters and their leak out of the cell. This allows to control the concentration gradient across the plasma membrane and to prevent possible toxic effects that could occur when the cytoplasmic concentration of catecholamines exceeds critical levels (Schuldiner, 1994, Masson et al., 1999). The storage process is mediated by specific vesicular transporters (Schuldiner et al., 1978, Erickson et al., 1992, Schuldiner, 1994, Masson et al., 1999). In mammals, there are two closely related vesicular monoamine transporter (VMAT) isoforms that are named VMAT-1 and VMAT-2. VMAT-1 is primarily present in endocrine and paracrine cells, while VMAT-2 is the predominant monoamine transporter in the nervous system (Masson et al., 1999). VMAT-2 is mainly expressed in dense core vesicles of axon terminals (Figure 3) (Nirenberg et al., 1997).

Monoamine vesicular transporters are relatively promiscuous when concerning to their substrates. They transport dopamine, NA, ADR, and 5-HT but they differ in substrate preference and affinity. Several vesicular transport complementary deoxyribonucleic acid (cDNA) have been cloned; these complementary DNAs reveal open reading frames predictive of proteins with 12 transmembrane domains (Masson et al., 1999). Reserpine inhibits the monoamine vesicular transport and ultimately leads to the depletion of catecholamines inside the nerve endings (von Euler, 1972, Masson et al., 1999).

The vesicular transport is driven by pH and potential gradients which are established by ATP-dependent proton pumps. The ATP-driven H^+ pump, on one hand, acidifies the vesicular lumen (ΔpH) and, on the other hand, generates a potential gradient ($\Delta\Psi$) (Schuldiner et al., 1978, Johnson and Scarpa, 1979). For every molecule taken in, two internal H^+ ions are extruded by VMAT-2 by an antiport process (Masson et al., 1999). Therefore, the intravesicular concentration of monoamines depends upon $\Delta\Psi$ and ΔpH (Njus et al., 1986, Rottenberg, 1986, Johnson, 1988, Schuldiner, 1994, Masson et al., 1999). In addition, the monoamine uptake is modulated by vesicle-associated heterotrimeric guanine nucleotide binding regulatory proteins (G proteins), $G_{\alpha 2}$ and $G_{\alpha q}$ (Ahnert-Hilger et al., 1998, Holtje et al., 2000, Holtje et al., 2003). G proteins control transmitter storage: the

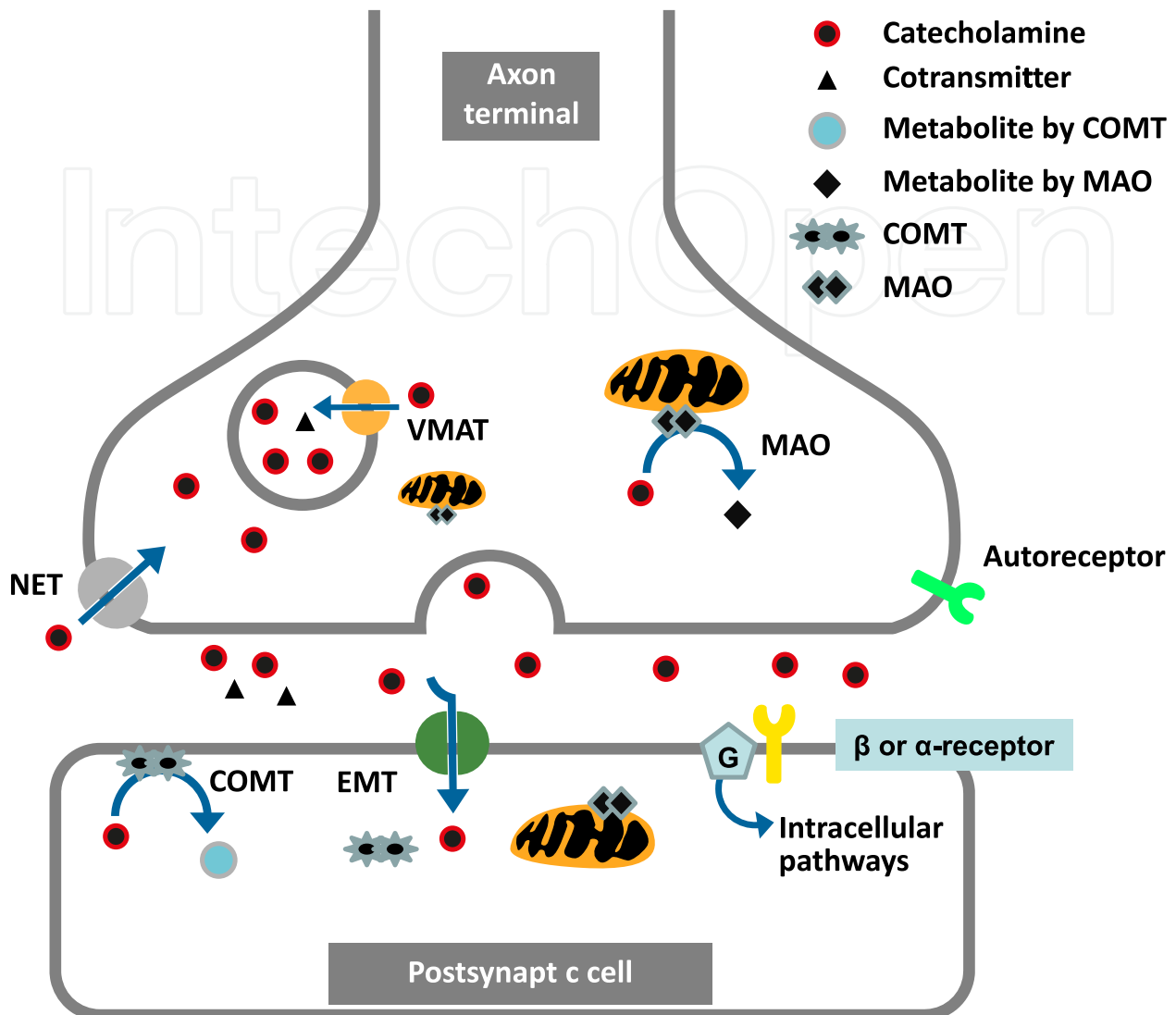


Fig. 3. Catecholamines in axon terminals. The axon terminal has neurotransmitter storage vesicles with catecholamines and cotransmitters. The vesicular monoamine transporter (VMAT) is responsible for the transport of catecholamines to the storage vesicles, maintaining their cytosolic concentration low. After catecholamine release to the synaptic cleft, catecholamines are reuptaken to the presynaptic terminal by noradrenaline transporter (NET), and/or taken to extraneuronal cells by the extraneuronal transporters. The most important extraneuronal transporter for catecholamines is extraneuronal monoamine transporter (EMT). Catecholamines are metabolized by intracellular enzymes. Monoamine oxidase (MAO) is located in the outer membrane of mitochondria in neurons and in extraneuronal cells. Catechol-*O*-methyl transferase (COMT) is located at extraneuronal cells. Both enzymes (COMT and MAO) are the main responsible for the metabolism of catecholamines. The enzymatic process leads to the formation of several metabolites. To exert their actions, the catecholamines and other neurotransmitters in the synaptic cleft bind to different pre- and postsynaptic receptors. Such binding leads to alterations in the postsynaptic cell and activation of intracellular pathways through G proteins. In presynaptic neurons, catecholamines bind to autoreceptors and activate feed-back responses that change their own release.

monoamines stored inside the vesicles represent the upstream signal that mediates the inhibition to further uptake through G proteins. In fact, biogenic amines, with the exception of ADR, lead to G protein-mediated inhibition; VMATs have a receptor-like area in their first luminal loop that senses the intravesicular concentration of monoamines (Brunk et al., 2006).

6. Release of catecholamines

Depolarization of the axonal terminal triggers the release of catecholamines into the cleft. The content of the vesicles, including enzymes, neurotransmitters, and hormones, is discharged to the exterior through a process termed exocytosis (Figure 3). Triggered exocytosis is the most common cellular mechanism for the release of polar molecules and it involves several mechanisms that include vesicle docking, priming, and fusion. It can be regulated or constitutive. In synaptic transmission, a synchronized mechanism provides the cells with a way for precisely timed release of molecules into the extracellular space (Pocock and Richards, 2006). The full sequence of steps by which the nerve impulse affects the release of transmitters from sympathetic neurons is not yet fully understood.

In the adrenal medulla, the triggering event to exocytosis is the release of acetylcholine by the preganglionic fibres; that acetylcholine binds to nicotinic receptors on chromaffin cells which produce a localized depolarization. In most types of cells, regulated exocytosis is triggered by the increase in cytosolic free calcium (Ca^{2+}) (Meir et al., 1999, Pocock and Richards, 2006). This cytosolic Ca^{2+} enters the cells from the extracellular medium, through the voltage-gated Ca^{2+} channels. The voltage-gated Ca^{2+} channels open following depolarization of the plasma membrane, making the Ca^{2+} able to diffuse into the cell down its electrochemical gradient. Ca^{2+} is also mobilized from intracellular stores (mainly the endoplasmic reticulum). As result, the intracellular free Ca^{2+} increases and it triggers the fusion of secretory vesicles with the plasma membrane. Ca^{2+} -triggered secretion involves the interaction of highly conserved molecular scaffolding proteins and leads to the docking of the vesicles (Aunis, 1998, Meir et al., 1999). As fusion continues, a pore is formed and it connects the extracellular space with the interior of the vesicle thus providing a pathway for vesicle content to diffuse into the extracellular space (Pocock and Richards, 2006) (Figure 3).

Other aspects concerning modulation of catecholamine transmission or secretion will be better addressed in a subsequent section where adrenoceptors and their role in augmenting or diminishing the synaptic release will be focused.

7. Neuronal transporters involved in catecholamine reuptake

Synaptic transmission involves the regulated release of transmitters into the synaptic cleft, where they interact with receptors that subsequently transduce the information. The removal of catecholamines from the cleft usually occurs by uptake either back to the presynaptic terminal or into the postsynaptic cell (Iversen et al., 1967, Axelrod and Kopin, 1969). A number of high-affinity transporters have been identified, namely for dopamine, NA, 5-HT, and several aminoacid transmitters (Masson et al., 1999). These transporters are members of an extensive family that share common structural motifs, particularly the putative 12-transmembrane helices (Bönisch and Brüss, 2006). The transporters show a similar configuration of intracellular and extracellular loops or related regions that contain

phosphorylation and glycosylation sites and intracellular located amino- and carboxyl-terminal residues (Eisenhofer, 2001). Dopamine is cleared by dopamine transporter whilst NA and ADR are cleared by NA transporter (NET) (Figure 3) (Trendelenburg, 1988, Apparsundaram et al., 1997, Eisenhofer, 2001). These plasma membrane transporters have higher substrate specificity than vesicular transporters and may be viewed as targets ("receptors") for several drugs such as cocaine (NET) or fluoxetine (5-HT transporter) (Bönisch and Brüss, 2006, Capela et al., 2008), but also MDMA (both) (Capela et al., 2009). Dopamine transporter and NET exhibit overlapping, yet distinct, substrate selectivity, translocation efficiency, and antagonist sensitivity (Buck and Amara, 1994, Giros et al., 1994, Pifl et al., 1996, Bönisch and Brüss, 2006). Other than the corresponding neurons, NET is also present in adrenal medulla, liver, and placenta, whereas dopamine transporter is present in the stomach, pancreas, and kidneys (Eisenhofer, 2001).

The neuronal NA uptake system was first reported in the spleen and heart (Axelrod et al., 1959, Axelrod et al., 1961). The availability of radiolabelled catecholamines enabled Axelrod and co-workers to examine the fate of these amines after their intravenous administration to laboratory animals (Axelrod et al., 1959, Axelrod et al., 1961). They observed a selective accumulation of radiolabelled ADR and NA in sympathetically innervated organs, which was dependent on intact sympathetic nerve terminals and it was inhibited by cocaine (Axelrod et al., 1959, Axelrod et al., 1961). It was shown that this accumulation occurred through the activity of NET.

The activity of neurotransmitter transporters, like NET and dopamine transporter, is dependent on intracellular sodium ion (Na^+) and chloride ion (Cl^-) concentrations. The Na^+ -gradient (extracellular concentration of Na^+ is higher) across the plasma membrane is the main driving force, dictating the direction of the transport of neurotransmitters and co-substrate ions (in these case Na^+). The normal direction is inwards (Masson et al., 1999, Sonders et al., 2005, Bönisch and Brüss, 2006). The negative intracellular membrane potential also contributes for the above mentioned driving force and it is mainly created by the potassium ion (K^+) gradient (Bönisch and Brüss, 2006).

Transport of NA by NET is saturated and it is characterized by a half-saturation constant or Michaelis constant (K_m) of about $0.8 \mu\text{M}$; ADR is transported by NET with a K_m of $2.8 \mu\text{M}$ in a maximum velocity two times lower than the transport of NA (Apparsundaram et al., 1997, Bönisch and Brüss, 2006). NET has a high affinity for NA and a somewhat lower affinity for ADR, while isoproterenol is not a substrate for this system (Eisenhofer, 2001, Bönisch and Brüss, 2006). The common structural requirement for uptake by NET is the presence of an ionisable nitrogen not incorporated in the aromatic ring system (Eisenhofer, 2001).

In amphibians, an ADR specific transporter has been identified, with characteristics distinct from those of dopamine transporter or NET (Apparsundaram et al., 1997). In mammals, the presence of an ADR specific transporter was not yet clarified, however *in situ* hybridization studies indicate the absence of both dopamine transporter and NET messenger ribonucleic acid (mRNA) in ADR-synthesizing neurons in the brainstem of adult rats (Lorang et al., 1994). Thus, as ADR is also cleared at these sites, these terminals might have a distinct catecholamine transporter from dopamine transporter or NET; as an alternative, ADR may act as an endocrine regulator that does not require rapid reuptake in those neurons (Lorang et al., 1994, Apparsundaram et al., 1997).

The importance of neuronal catecholamine transporters in maintaining vesicular levels of catecholamines is well illustrated in mutant mice lacking NET. NET is a high-affinity system, relatively selective for NA, with a low maximum rate of uptake, and it is important in maintaining releasable stores of NA (Bönisch and Brüss, 2006). The knockout NET mice showed depletion of NA intraneuronal stores, lack of inhibition of neuronal amine synthesis, protracted clearance, and elevated NA extracellular levels (Wang et al., 1999). Interestingly, in cyclic voltammetry experiments with the knockout NET mice, the rate of NA clearance was decreased by only six fold when compared to the rate of wild-type animals (Xu et al., 2000). The knockout NET mice behave like wild-type animals treated with antidepressant drugs and are hyperresponsive to locomotor stimulation with psychostimulants (Xu et al., 2000).

In summary, the neuronal reuptake process aims to assure constant and high levels of neurotransmitters in the releasing neuron and low concentrations in the cleft (Eisenhofer, 2001, Bönisch and Brüss, 2006). It works as an integrated part of the neurotransmitter recycling process, since in addition to their *de novo* synthesis, the stores of monoamines in the terminal portions of the neural fibres are also replenished by their active transport back to the terminals. Moreover, the reuptake system contributes to the degradation of catecholamines since the metabolizing enzymes are found intracellularly (Westfall and Westfall, 2006). Furthermore, the reuptake of catecholamines maintains the concentration gradient within the neuronal vesicles. Two distinct neuron carrier-mediated transport systems are involved: one across the axoplasmic membrane from the extracellular fluid to the cytoplasm, NET or dopamine transporter, and the other from the cytoplasm into the storage vesicles, the VMAT-2 (Bloom, 2006). The removal of catecholamines from the cytoplasm into the storage system by VMAT-2 acts as an amplification step for the overall uptake process developed by NET or dopamine transporter (Schuldiner, 1994, Sonders et al., 2005). In a broader sense, catecholamine transporters function as part of an integrated system where catecholamine synthesis, release, uptake, and metabolism are regulated in a coordinated fashion (Eisenhofer, 2001). Therefore, neuronal catecholamine transporters function not only as part of metabolizing systems, but perhaps more importantly, as part of the recycling system, operating in series with VMAT-2 to maintain catecholamine neuronal stores (Eisenhofer, 2001) (Figure 3).

8. Catecholamine extraneuronal transporters

Catecholamines are also taken up by extraneuronal transporters. A low affinity, high capacity uptake for NA and ADR in the isolated perfused rat heart was found by Iversen (Iversen, 1963, 1965b,a). Iversen observed that radiolabelled [^3H]-NA in the isolated heart entered into at least two intracellular pools with distinct rate constants. In fact, [^3H]-NA entered one pool approximately seven-times faster when compared to the other pool (Iversen, 1963). In 1967, Malmfors demonstrated, by fluorescence microscopy, that the myocytes were responsible for an uptake process (Malmfors, 1967). After these discoveries, the neuronal NA uptake system was designated as “uptake-1” and the extraneuronal transport system as “uptake-2” (Iversen, 1965b). The extraneuronal uptake of catecholamines is mediated by organic cation transporters (OCTs), that include the classic corticosterone-sensitive extraneuronal monoamine transporter and traditionally named “uptake-2” that nowadays is called OCT3 (Eisenhofer, 2001, Schömig et al., 2006). Other members of this family are: OCT1 and OCT2.

All three OCTs can transport catecholamines in addition to a wide variety of other organic acids, including 5-HT, histamine, choline, spermine, guanidine, and creatinine (Eisenhofer, 2001). The affinity of extraneuronal monoamine transporter for NA is rather low ($K_m > 0.5$ mM), which is compensated by its high capacity (high turnover number). The transport efficiency of OCT1 and OCT2 for dopamine, NA, ADR, and 5-HT is usually low (Schömig et al., 2006).

OCTs share common features. A single positive charge is required to be an OCT substrate, while uncharged, doubly charged or negatively charged substances are not transported by OCTs (Schömig et al., 2006). A decrease in extracellular pH and the depolarization of the cytoplasmic membrane will reduce the OCT activity. The OCT mediated transport is independent of Na^+ and Cl^- gradients and all three OCTs are transporters, not just channels, as shown by trans-stimulation experiments. OCT1, OCT2 or extraneuronal monoamine transporter may mediate electrogenic uniport of a substrate or electroneutral exchange of two substrates (antiport). The OCTs are relatively resistant to the inhibitors of the neuronal transporters such as desipramine (Eisenhofer et al., 1991, Schömig et al., 2006), but are inhibited by steroids, such as corticosterone, and the *O*-methylated metabolites of catecholamines, like normetanephrine and metanephrine (Eisenhofer, 2001, Costa et al., 2009a, Nissinen and Männistö, 2010). Other compounds, like GF120918, were shown to inhibit extraneuronal monoamine transporter at low concentrations in isolated rat cardiomyocytes (Costa et al., 2009a).

Extraneuronal monoamine transporter (OCT3) is expressed in many but not all tissues. The expression varies greatly between organs and during organ development (e.g. in placenta). Consistently, high expression has been reported for placenta and heart (Eisenhofer, 2001). Other reports indicate high extraneuronal monoamine transporter expression in the area postrema (Haag et al., 2004, Vialou et al., 2004) and very low in the kidneys (Eisenhofer, 2001). Interestingly, extraneuronal monoamine transporter expression has been also suggested to occur in neurons (Kristufek et al., 2002, Shang et al., 2003).

For NA, the uptake by NET is more relevant for signal termination than extraneuronal uptake. It has been estimated that the sympathetic nerves remove approximately 87% of released NA by NET, while 5% is removed by extraneuronal monoamine transporter. The remainder 8% is diffused into the circulation (Eisenhofer, 2001). This data, however, does not necessarily reflect the relative importance of the two processes. The proximity of the neuronal transporters to the location of catecholamine release (when compared to extraneuronal transporters) implies that the transmitter removed by extraneuronal transport has a higher duration and wider range of action. Extraneuronal uptake, therefore, may be particularly important for the removal of neuronal released catecholamines in tissues where high concentrations of adrenergic receptors exist (Eisenhofer, 2001). The clearance of circulating catecholamines is primarily mediated by non neuronal mechanisms, with liver and kidney accounting for over 60% (Eisenhofer, 2001, Westfall and Westfall, 2006). As stated above, most NA released by sympathetic nerves is removed by neuronal reuptake, which is in contrast with that of ADR. ADR when secreted directly into the bloodstream from the adrenal medulla is predominantly inactivated by extraneuronal uptake and metabolism. ADR has lower affinity for neuronal uptake than NA and consequently the neuronal process contributes approximately 50% less for its elimination when compared with NA (Iversen, 1965a). In contrast, ADR is removed by extraneuronal uptake 2-3 times more efficiently than NA (Iversen, 1965b, Eisenhofer et al., 1992b).

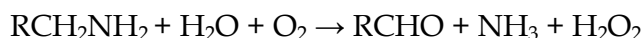
In short, when compared to NET, extraneuronal monoamine transporter exhibits lower affinity (i.e. higher K_m) for catecholamines, favours ADR and isoproterenol over NA or dopamine, and shows a higher maximum rate for catecholamine uptake (i.e. higher maximum velocity) (Iversen, 1965a, b, Eisenhofer et al., 1992b, Eisenhofer, 2001, Schömig et al., 2006). Extraneuronal monoamine transporter is not Na^+ -dependent and displays a completely different profile of pharmacological inhibition (Schömig et al., 2006, Westfall and Westfall, 2006). Thus, in contrast to the importance of NET in the clearance of neuronal released catecholamines, clearance of circulating catecholamines is predominantly made by non-neuronal mechanisms (Eisenhofer, 2001).

9. Metabolism and action termination

The pharmacological actions of NA and ADR are terminated by: (i) reuptake into nerve terminals by NET; (ii) uptake at extraneuronal sites by extraneuronal monoamine transporter, OCT1, or OCT2; and (iii) metabolic transformation. Catecholamines undergo a complex metabolic fate, mediated by several enzymes, including aldehyde reductase, aldose reductase, aldehyde dehydrogenase, alcohol dehydrogenase, catechol-*O*-methyltransferase (COMT), dopamine β -hydroxylase, monoamine oxidase (MAO) type A and B, monoamine-preferring phenolsulfotransferase, and PNMT (Dooley, 1998, Goldstein et al., 2003). *In vivo*, two of the most important enzymes responsible for the metabolic transformation of catecholamines are MAO and COMT (Westfall and Westfall, 2006).

9.1 Monoaminoxidase metabolism

MAO was first described as tyramine oxidase by Mary Hare-Bernheim in 1928, since it catalyses the oxidative deamination of tyramine. This enzyme was found to oxidize several monoamines, including catecholamines, i.e. dopamine, NA, and ADR, and also 5-HT. MAO [amine: oxygen oxidoreductase (deaminating)] catalyses the following reaction:



MAO acts on primary amines and also on some secondary and tertiary amines (Nagatsu, 1991). It is located in the outer membrane of mitochondria (Schnaitman et al., 1967) both in neuronal and extraneuronal cells (Trendelenburg, 1988) (Figure 3). It is a flavo-protein, with flavin adenine dinucleotide as cofactor (Kearney et al., 1971). Two forms of MAO exist, i.e. MAO-A and MAO-B (Johnston, 1968). In humans, MAO-A is abundant in the brain, liver, and in the syncytiotrophoblast layer of term placenta, whereas liver, lungs, platelets, lymphocytes, osteocytes that surround the blood vessels, and the intestine are rich in MAO-B (Abell and Kwan, 2001, Nagatsu, 2004). In the human brain, MAO-A is located in the catecholamine-containing regions, with the highest levels being found in *locus ceruleus*. MAO-B is prominent in the dorsal *raphe nuclei*, which are known to have serotonergic neurons. MAO-B is also present in the posterior hypothalamus and in glial cells (Abell and Kwan, 2001, Nagatsu, 2004).

5-HT, NA, and ADR are preferential substrates for MAO-A, and clorgyline and Ro 41-1049 are MAO-A inhibitors (Johnston, 1968, Kitaichi et al., 2010); MAO-B prefers β -phenylethylamine as a substrate and it is inhibited by deprenyl and lazabemide (Knoll et al., 1978, Kitaichi et al., 2010). Dopamine, tyramine, and tryptamine are oxidized with equal affinity by MAO-A and MAO-B (Glover et al., 1977). Pargyline inhibits both MAO-A and

MAO-B (Blaaha et al., 1996, Carvalho et al., 2001, Carmo et al., 2003, Duarte et al., 2004). MAO-A appears to be the main enzyme in the metabolism of 5-HT and NA, while the location of MAO-B, abundant in serotonergic neurons, remains under debate (Abell and Kwan, 2001).

In the brain of MAO-A-deficient mouse pups, NA levels increased up to two-fold, while a small increase in dopamine levels was observed. Borderline mental retardation and abnormal behavioural are observed in humans with selective MAO-A deficiency. Severe mental retardation in patients with combined MAO-A/MAO-B deficiency and Norrie disease are also observed (Lenders et al., 1996). Human males from a Dutch family with a complete deletion of the *mao-a* gene in the X chromosome were reported to show abnormal aggressive behaviour (Brunner et al., 1993), as observed in adult mice (Cases et al., 1995). MAO-A-deficient adult male mice show enhanced aggressive behaviour and enhanced emotional learning, probably due to elevated levels of 5-HT in the brain in the pup stage (Cases et al., 1995, Kim et al., 1997). Furthermore, MAO-A knockout mice exhibited a dramatic reduction of defensive and fear-related behaviours in the presence of predator-related cues, such as predator urine or an anaesthetized rat (Godar et al., 2010).

All regions of the brain of MAO-B-deficient mouse pups showed increased levels of β -phenylethylamine (Lenders et al., 1996), especially in striatum and prefrontal cortex (Bortolato et al., 2009). The MAO-B knockout mice show behavioural disinhibition and decrease anxiety-like responses partially through a regional increase in β -phenylethylamine levels (Bortolato et al., 2009). In contrast to the borderline mental retardation and abnormal behavioural phenotype in subjects with selective MAO-A deficiency and the severe mental retardation in patients with combined MAO-A/MAO-B deficiency and Norrie disease, the MAO-B-deficient human subjects neither exhibited abnormal behaviour nor mental retardation except increased reactivity to stress (Grimsby et al., 1997). The subjects with the *mao-b* gene deletion have complete absence of platelet MAO-B activity and have increased brain levels and urinary excretion of β -phenylethylamine (Lenders et al., 1996). In fact, the inhibition of MAO-B indicates that this form of MAO has a lower contribution to the metabolism of endogenous catecholamines and of their *O*-methylated metabolites (Eisenhofer and Finberg, 1994).

9.2 Catechol-O-methyl transferase metabolism

Another important catecholamine-metabolizing enzyme (COMT) was first described and purified by Axelrod (Axelrod, 1958, Axelrod and Tomchick, 1958) (Figure 3). One single gene for COMT codes for both soluble COMT and membrane-bound COMT. That gene has different transcription starting sites. COMT is an intracellular enzyme, the most abundant form being the soluble COMT with a minor fraction as membrane bound COMT (Mannisto and Kaakkola, 1999). COMT catalyses the transfer of the methyl group of *S*-adenosyl-L-methionine to one of the hydroxyl groups in the catechol in the presence of magnesium ion (Mg^{2+}) (Mannisto and Kaakkola, 1999). COMT is widely distributed throughout the body. High levels of COMT are found in the liver, proximal tubular epithelial cells of the kidneys, and other extraneuronal cells, namely adrenomedullary chromaffin cells (Eisenhofer et al., 1998, Mannisto and Kaakkola, 1999). However, little or no COMT is found in sympathetic neurons. In the presynaptic terminals of the brain, no significant COMT is detected, but it is reported in some postsynaptic neurons and glial cells. The physiological substrates of

COMT include L-3,4-dihydroxyphenylalanine, all three endogenous catecholamines (dopamine, NA, and ADR), their hydroxylated metabolites, catecholestrogens, ascorbic acid, and dihydroxyindolic intermediates of melanin (Mannisto and Kaakkola, 1999, Nissinen and Männistö, 2010).

In COMT knockout mice, minor behaviour changes were detected and the neurochemistry of catecholamines in the brain is virtually unaltered (Gogos et al., 1998). Mutant mice showed sexually dimorphic and region-specific changes of dopamine levels, notably in the frontal cortex. Mutant male mice have almost a three-fold increase of dopamine levels in the frontal cortex, while no changes were observed in the striatum or hypothalamus. In mutant female mice no alterations in dopamine levels were observed when compared to the wild type (Gogos et al., 1998). Despite the complete lack of *comt* gene in homozygous mice, residual HVA levels were detectable in several brain areas, revealing a possible and still unidentified methylation pathway in the brain (Gogos et al., 1998). Female knockout mice showed impaired emotional reactivity, while heterozygous males were more aggressive (Gogos et al., 1998). The work by Gogos and colleagues suggested that the importance of COMT in the emotional and social behaviour has probably been undervalued and the complete lack of COMT can be partially compensated, at least in mice (Gogos et al., 1998).

9.2.1 The cross-talk between catecholamine catabolic enzymes and transporters

Of relevance, the main mechanism that reduces the life span of catecholamines in the extracellular space is their uptake by active transport and not their enzymatic metabolism. It has been shown that NET inhibitors (e.g., cocaine, imipramine, and desipramine) potentiate the effects of the neurotransmitters, while inhibitors of MAO and COMT have relatively little immediate effect (Gonçalves et al., 1989, Eisenhofer, 1994, Eisenhofer and Finberg, 1994, Friedgen et al., 1994, Blaha et al., 1996, Friedgen et al., 1996). Early studies in isolated tissues showed that the inhibition of MAO or COMT could limit the ability of neuronal and extraneuronal uptake to clear extracellular catecholamines (Furchgott and Garcia, 1968, Belfrage et al., 1977). More recent *in vivo* studies however indicate that inhibition of one enzyme under normal physiological conditions has little effect on the overall catecholamine clearance and on their extracellular or circulating levels (Eisenhofer, 1994, Eisenhofer and Finberg, 1994, Friedgen et al., 1994, Blaha et al., 1996, Friedgen et al., 1996). Only when MAO and COMT are both inhibited or subjected to saturating concentrations of substrate, the clearance of catecholamines is significantly impaired (Eisenhofer, 1994, Friedgen et al., 1996). To confirm the redundancy of metabolizing systems, in MAO-A-deficient humans or rats a marked decrease in deaminated catecholamine metabolites and a concomitant marked elevation of *O*-methylated amine metabolites occur (Eisenhofer and Finberg, 1994, Lenders et al., 1996). These neurochemical changes are only slightly exaggerated in patients with combined lack of MAO-A and MAO-B (Lenders et al., 1996).

The activity of metabolizing enzymes can influence the catecholamine net transport and the extracellular levels of catecholamines. Moreover, the metabolizing enzymes are always required for the irreversible chemical alteration of catecholamines following neuronal or extraneuronal uptake. Nevertheless, Trendelenburg stated that understanding the

interactions and the functions of the active transport and metabolizing processes is very relevant as they function as “pump and leak systems with enzyme(s) inside” (Trendelenburg, 1988, 1990).

An integrated vision explains the results found *in vivo*. In fact, the presence of only one catecholamine-metabolizing enzyme, MAO, in neuronal systems would lead to the assumption that inhibition of this enzyme would impair the catecholamine’s neuronal uptake by changing the concentration gradient. However, the additional presence of VMAT-2 maintains the axoplasmic catecholamine levels low. Furthermore, VMAT-2 has higher affinity for NA than MAO, which implies that over 70% of the recaptured NA through NET is sequestered into storage vesicles before being metabolized (Bloom, 2006). Therefore, under normal physiological conditions, the inhibition of MAO has little effect (Graefe and Trendelenburg, 1970, Graefe and Henseling, 1983). Rather than impairing the catecholamine uptake, short-term inhibition of intraneuronal MAO leads to higher retention of recaptured transmitter by VMAT-2 and, thus, an apparent increase in the net uptake of the transmitter (Furchgott and Garcia, 1968). In the long term, if vesicular storage capacity is overwhelmed, inhibition or saturation of intraneuronal MAO can lead to a decrease in neurotransmitter’s uptake (Trendelenburg et al., 1972).

9.3 The metabolites of catecholamines

Complex and dynamic processes are involved in the metabolism of catecholamines, either intracellularly or even after their release into the extracellular fluid. In both neuronal and extraneuronal metabolizing systems, the inactivation of catecholamines occurs in a coordinated fashion, with uptake being followed by metabolism (Graefe and Henseling, 1983). Most of the metabolism of catecholamines takes place in the same cells where they are produced, even before their exocytotic release (Figure 4). Dihydroxyphenylglycol (DHPG, 3,4-dihydroxyphenylethylene glycol) constitutes the main NA metabolite before NA release or reuptake. DHPG is also a deaminated metabolite of ADR (Eisenhofer and Finberg, 1994, Goldstein et al., 2003). DHPG is formed from NA in the cytoplasm of sympathetic nerves by sequential deamination of NA by MAO to form dihydroxyphenylglycoaldehyde. This aldehyde is reduced by aldehyde reductase or aldose reductase to form DHPG or it is oxidized by aldehyde dehydrogenase to form 3,4-dihydroxymandelic acid. DHPG diffuses rapidly across the membrane of the cell into the extracellular fluid and into extraneuronal cells. In the extraneuronal cells, DHPG is metabolized by COMT to form 3-methoxy-4-hydroxy-phenylethylene glycol, or it overflows into the bloodstream (Eisenhofer and Finberg, 1994, Goldstein et al., 2003). Also, the decrease in plasma concentrations of endogenous DHPG and 3,4-dihydroxyphenylacetic acid after inhibition of MAO-A, but not MAO-B, corroborates that NA is a better substrate for the A isoform (Eisenhofer and Finberg, 1994).

COMT has a substantial importance in the metabolism of the deaminated metabolites of NA and dopamine, as corroborated by the increase of plasma levels of 3,4-dihydroxyphenylacetic acid and DHPG after COMT inhibition (Eisenhofer and Finberg, 1994). Alternatively, COMT can catalyse the *O*-methylation of NA to normetanephrine, of ADR to metanephrine, of L-3,4-dihydroxyphenylalanine to 3-methoxytyrosine, and of dopamine mainly to 3-methoxytyramine (Eisenhofer and Finberg, 1994, Goldstein et al., 2003).

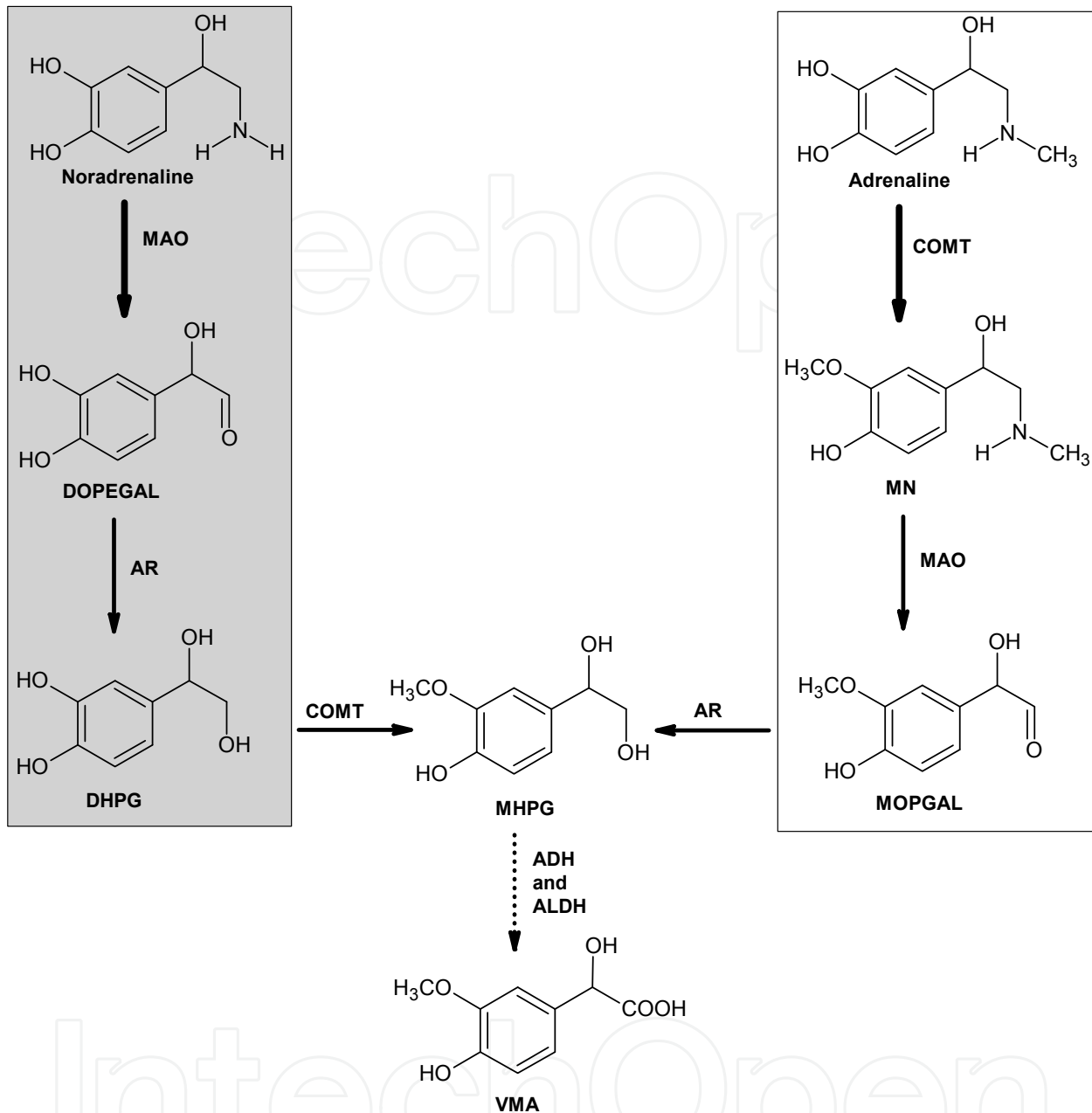


Fig. 4. Representative pathway of the most common metabolic pathways of adrenaline (ADR) and noradrenaline (NA). ADR is synthesized from NA through phenylethanolamine N-methyltransferase (PNMT) catalysis. NA is metabolized preferably by monoamine oxidase (MAO) to dihydroxyphenylglycoaldehyde (DOPEGAL) that is reduced by aldehyde reductase (AR) to form dihydroxyphenylethylene glycol (DHPG). ADR is preferably metabolized by catechol-O-methyltransferase (COMT) resulting in metanephrine (MN). MN can be further metabolized by MAO to 3-methoxy-4-hydroxyphenyl-glycoaldehyde (MOPGAL) and by aldehyde reductase (AR) to form 3-methoxy-4-hydroxy-phenylethylene glycol (MHPG). MHPG is further metabolized and can be transformed to vanillylmandelic acid (VMA) by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). VMA is the main catecholamine metabolite in urine (adapted from reference Goldstein et al., 2003).

In adrenal chromaffin cells, as they contain both MAO and COMT, the deamination and *O*-methylation coexist. The COMT present in chromaffin cells is mainly membrane bound COMT while soluble COMT is found in the majority of other tissues (namely liver and kidneys). Membrane bound COMT has a higher affinity for catecholamines than the soluble COMT (Roth, 1992). As a result, in adrenal chromaffin cells, leakage of NA and ADR from storage granules leads to substantial intracellular production of the *O*-methylated metabolites, normetanephrine and metanephrine. In humans, about 93% of circulating metanephrine and between 25% to 40% of circulating normetanephrine result from the catecholamines metabolized within adrenal chromaffin cells (Eisenhofer et al., 2004).

In most cells, the *O*-methylated compounds that contain amine groups undergo further metabolic alterations by MAO. Deamination of 3-methoxytyramine yields HVA and deamination of normetanephrine and metanephrine yields 3-methoxy-4-hydroxy-phenylethylene glycol. 3-Methoxy-4-hydroxy-phenylethylene glycol in human plasma is a very important metabolite with multiple sources, including (i) deamination of normetanephrine after its cellular uptake; (ii) *O*-methylation of DHPG after its uptake from the circulation; and (iii) *O*-methylation of DHPG after its uptake from the interstitial fluid but before its entrance into the circulation. Of the previously mentioned sources, the most relevant is the last (Eisenhofer and Finberg, 1994). The fate of circulating 3-methoxy-4-hydroxy-phenylethylene glycol is complex and includes sulfation, glucuronidation, urinary excretion, and specially conversion to methoxy-4-hydroxymandelic acid [generally, called vanillylmandelic acid (VMA)] through the hepatic sequential oxidation of circulating 3-methoxy-4-hydroxy-phenylethylene glycol by alcohol dehydrogenase and aldehyde dehydrogenase (Goldstein et al., 2003). In fact, the major product of urinary excretion and also an important plasma metabolite of adrenergic catecholamines is VMA (Goldstein et al., 2003) (Figure 4).

The formation of normetanephrine occurs after NA extraneuronal uptake and metabolism. Due to the importance of the reuptake and intraneuronal deamination of endogenously released NA, plasma levels of normetanephrine are lower than those of DHPG, despite the similar plasma clearance of these two compounds. The rate of extra adrenal production of normetanephrine, although low, still provides a unique marker of NA extraneuronal metabolism. Accordingly, during MAO-A inhibition, normetanephrine and metanephrine levels increase as COMT becomes more relevant in the metabolism of NA (Eisenhofer and Finberg, 1994).

As stated, adrenomedullary chromaffin cells contain both MAO and COMT and, in agreement with the different affinities between extraneuronal and neuronal uptake, ADR is less metabolized by MAO than NA, but it is a better substrate for COMT (Paiva and Guimarães, 1978, Eisenhofer and Finberg, 1994). Because of these differences, extraneuronal uptake and *O*-methylation clear more circulating ADR than NA (Axelrod et al., 1959, Eisenhofer, 2001, Goldstein et al., 2003) and metanephrine is the major metabolite of ADR (Axelrod et al., 1959, Goldstein et al., 2003).

In cells that contain monoamine-preferring phenolsulfotransferase, the non-acid metabolites, methoxytyramine, normetanephrine, metanephrine, and 3-methoxy-4-hydroxy-phenylethylene glycol undergo extensive sulphate-conjugation. The urinary metabolites, resulting from monoamine-preferring phenolsulfotransferase metabolism are usually 3-

methoxy-4-hydroxy-phenylethylene glycol-SO₄, normetanephrine-SO₄, and metanephrine-SO₄ (Eisenhofer et al., 2004).

Glucuronides of methoxytyramine, normetanephrine, metanephrine, and 3-methoxy-4-hydroxy-phenylethylene glycol are also formed and they may be excreted in the bile. When these glucuronides enter into the circulation, they are eliminated in the urine (Eisenhofer et al., 2004).

Of relevance, the oxidative deamination of catecholamines by MAO leads to another relevant product, hydrogen peroxide (H₂O₂) which subsequently may be converted into the highly reactive hydroxyl radical (HO•) (Valko et al., 2007), that may cause oxidative stress-related damage (Carvalho et al., 2001, Duarte et al., 2004, Vaarmann et al., 2010, Costa et al., 2011).

9.3.1 Levels of catecholamines and their metabolites in the plasma and urine

Catecholamines enter the plasma mainly after their leakage from the synaptic cleft to the circulation or after the secretion of catecholamines by the adrenal medulla (Esler et al., 1990, Goldstein et al., 2003). The bulk of NA in the plasma results mainly from sympathetic overflow, while the majority of plasma ADR is formed by adrenal medulla secretion (Esler et al., 1990, Goldstein et al., 2003). The metabolites of NA and ADR also spill into the plasma after intraneuronal metabolism (namely oxidative deamination by MAO) or extraneuronal metabolism (preferentially COMT-methylation), since the majority of the metabolism occurs even before the catecholamines reach the circulation (Goldstein et al., 2003). The most abundant human plasma metabolites are VMA, 3-methoxy-4-hydroxy-phenylethylene glycol and its sulphate metabolite (Goldstein et al., 2003). The main human plasma catechols are the three catecholamines, their precursor, L-3,4-dihydroxyphenylalanine, and their deaminated metabolites, 3,4-dihydroxyphenylacetic acid from dopamine, and DHPG from NA (Goldstein et al., 2003) (Figure 4).

In humans, the sympathetic nerves of kidneys and skeletal muscles are the major sources of plasma NA, each contributing with approximately 25% of the total (Esler et al., 1984a, Esler et al., 1984b). The sympathetic innervations of the human heart, skin, gastrointestinal tract, lungs, and liver are responsible for a minor percentage of total plasma NA, but equally important (Esler et al., 1984a, Esler et al., 1984b, Goldstein et al., 1988, Esler and Kaye, 2000).

The rate of NA entrance into the arterial plasma ("total body spillover") can be measured and in healthy people averages 0.3 to 0.5 µg/min (Goldstein et al., 2003). Key players in the rate of NA spillover include the rate of NA release, and consequently nerve firing velocity and density, but also regional blood flow, capillary permeability, neuronal NA uptake, and NA metabolism (Esler et al., 1990). These key aspects have to be taken into account when interpreting NA overflow as an index of sympathetic activity. In fact, when comparing the levels of NA release into interstitial fluid to circulation with NA spillover, the NA release into interstitial fluid in kidneys averages 3 times, in skeletal muscle 12 times, and in the heart over 20 times the NA spillover, due to the efficient local neuronal reuptake of NA. (Goldstein et al., 2003).

The majority of plasma ADR in physiological conditions results from the stimulation of adrenal medulla (Goldstein et al., 2003); it is assumed that ADR overflow from adrenergic nerves is not very relevant (Esler et al., 1990). The only exception is the heart, which at very

high stimulation rates can contribute significantly for ADR spillover into the plasma (Peronnet et al., 1988, Johansson et al., 1997).

Plasma levels of ADR in healthy volunteers at rest are as low as 30 pM, while NA reaches 1 nM (Wheatley et al., 1985, Goldstein et al., 2003). Spillover of ADR to arterial plasma in healthy resting humans is typically 30-100 pg/mL, while it can reach 200-600 ng/mL for NA (Esler et al., 1990). Any alteration in the metabolism of catecholamines or disruption of their transport mechanisms might lead to abnormal high concentrations of these substances (Lameris et al., 2000). Elevated levels of circulating and interstitial catecholamines are found in arrhythmias, myocardial necrosis (Lameris et al., 2000, Behonick et al., 2001), heart failure, (Esler and Kaye, 2000), myocardial ischemia (Lameris et al., 2000, Akiyama and Yamazaki, 2001, Killingsworth et al., 2004, Kuroko et al., 2007), exercise (Kjaer, 1998), pheochromocytoma (Gerlo and Sevens, 1994), hypoglycaemia, haemorrhagic hypotension, circulatory collapse, distress (Goldstein et al., 2003), and cirrhosis (Esler et al., 1990). The administration of amphetamines can also lead to peaks of plasmatic levels of biogenic catecholamines. In fact, after high doses of *d*-amphetamine in rats, levels of plasmatic ADR and NA reached 146 nM and 418 nM, respectively (Carvalho et al., 1997).

The levels of urinary and plasma catecholamine or metabolites can also indicate a higher sympathetic overflow or metabolism impairment (Esler et al., 1990, Brunner et al., 1993, Lenders et al., 1996, Behonick et al., 2001, Goldstein, 2003). In fact, the measurement of DHPG gives important information of NA sympathetic nerve neuronal uptake and turnover (Esler et al., 1990, Goldstein et al., 2003). The DHPG values reflect the sum of vesicular leakage, NA deamination (which is mainly neuronal) and reuptake (Eisenhofer et al., 1992a, Goldstein et al., 2003). DHPG plasma levels can reach 4.7 nM in healthy volunteers (Goldstein et al., 2003).

Formation of normetanephrine in the body occurs after extraneuronal uptake and metabolism of NA in the sympathetic terminals, as well as from the *O*-methylation of NA within the adrenal medulla by COMT. Because of the high importance of reuptake and intraneuronal deamination of NA, plasma levels of normetanephrine (~0.3 nM) are significantly lower than those of DHPG (Goldstein et al., 2003).

O-Methylation is the main metabolic pathway of ADR in man (Labrosse et al., 1958). Metanephrine constitutes a major metabolite of ADR before its release into the extracellular fluid (Labrosse et al., 1958, Axelrod et al., 1959). Plasma metanephrine levels are roughly the same as plasma normetanephrine levels, although the levels of plasma NA are about 5- to 10-fold higher than the levels found for ADR. The levels of metanephrine result from the high rate of production of ADR in adrenomedullary chromaffin cells, the metabolism of adrenomedullary catecholamines by COMT (Goldstein et al., 2003), and the relatively high affinity of COMT for circulating ADR (Paiva and Guimarães, 1978, Eisenhofer and Finberg, 1994).

As already stated, 3-methoxy-4-hydroxy-phenylethylene glycol in human plasma has multiple sources, the main being *O*-methylation of DHPG after its uptake from the interstitial fluid but before its entrance into the circulation. The metabolic fate of the circulating 3-methoxy-4-hydroxy-phenylethylene glycol is complex and includes sulfation, glucuronidation, and specially conversion to VMA (Goldstein et al., 2003). 3-Methoxy-4-hydroxy-phenylethylene glycol and VMA constitute the major non sulphate catecholamine metabolites, reaching plasmatic values of 30 and 20 nM, respectively (Goldstein et al., 2003).

L-3,4-dihydroxyphenylalanine is the precursor of catecholamines and the product of the rate-limiting step of catecholamine synthesis. L-3,4-dihydroxyphenylalanine therefore occupies a crucial role in the catecholaminergic system. In humans, plasma levels of L-3,4-dihydroxyphenylalanine exceed those of NA about 10-fold, reaching 8.9 nM (Goldstein et al., 2003).

Some catecholamine storage vesicle elements are released during exocytosis like chromogranin A, DhB, and neuropeptide Y. Their plasma values can be used as an index of the neuroendocrine system activation (Esler et al., 1990).

All catecholamines are ultimately excreted in urine, either in their native form or as metabolites (Esler et al., 1990, Goldstein et al., 2003). Nevertheless, in urine, only a small fraction of catecholamines is present in their unaltered form (Esler et al., 1990) and NA urinary excretion represents only 1-2% of the total NA synthesized. VMA is the major catecholamine metabolite excreted in the urine and 33 μ M of VMA can be eliminated each 24 h (Gerlo and Sevens, 1994). The sulphate derivatives of 3-methoxy-4-hydroxyphenylethylene glycol, normetanephrine, and metanephrine are the bulk of the other urinary metabolites (Eisenhofer et al., 2004). The use of urinary excretion levels of catecholamines and/or of their metabolites to estimate total body catecholamine turnover and plasma levels has to be very cautious. Urinary excretion depends on renal blood flow and renal function, thus it has some inter-individual variations (Fluck, 1972).

9.4 Another pathway to degrade catecholamines

The metabolization of catecholamines is generally attained either by deamination via MAO or *O*-methylation by COMT. However, the chemical alteration of catecholamines by other enzymes or through oxidative processes is also a probable pathway to the chemical alteration of the transmitters (Richter, 1940)(Figure 5). When the enzymes dealing with the catabolism of catecholamines are unable to cope efficiently, their levels rise and catecholamines can undergo oxidation. The oxidation rate is faster under enzymatic or metal catalysis (Heacock, 1959, Bindoli et al., 1992, Foppoli et al., 1997), in the presence of the superoxide anion ($O_2^{\bullet-}$) or high pH (West, 1947, Spencer et al., 1995, Costa et al., 2007). Although at physiological pH, the oxidation of catecholamines seems to occur very slowly, it has been found to occur *in vivo* namely in the septic shock (Macarthur et al., 2000). The oxidation of catecholamines ultimately produces a family of indole semiquinonic/quinonic species usually termed catecholaminochromes because of their orange-reddish colour (Heacock and Mahon, 1958). The oxidation of aqueous extracts of mammal suprarenal capsules was first reported by Vulpian (Vulpian, 1856). The oxidation products of ADR were crucial for the indirect quantification of ADR for many years, since adrenolutin and adrenochrome are easily detected by UV/VIS or by fluorometric methods (Annrsten et al., 1949, Fischer and Bacq, 1950, Ludemann et al., 1955, Remião et al., 2003, Ochs et al., 2004).

Generally speaking, catecholamines may be oxidized to an unstable *o*-semiquinone that, after deprotonation and loss of a second electron, gives rise to the corresponding *o*-quinone. For ADR, at physiological pH, partial deprotonation of the amine group of the side chain leads to an irreversible 1,4-intramolecular cyclization, a reaction that occurs through nucleophilic attack of the nitrogen atom at position 6 of the quinone ring, to form "leucoadrenochrome"; leucoadrenochrome is subsequently oxidized to form adrenochrome

(Heacock and Mahon, 1958, Bindoli et al., 1992, Bindoli et al., 1999). This indole is often represented as a zwitterionic structure in aqueous solutions (Heacock and Mahon, 1958, Remião et al., 2003, Costa et al., 2007) (Figure 5). In summary, the oxidation of catecholamines occurs through two-stages whereby a total of four electrons is removed and an indole is formed by cyclization (Costa et al., 2011).

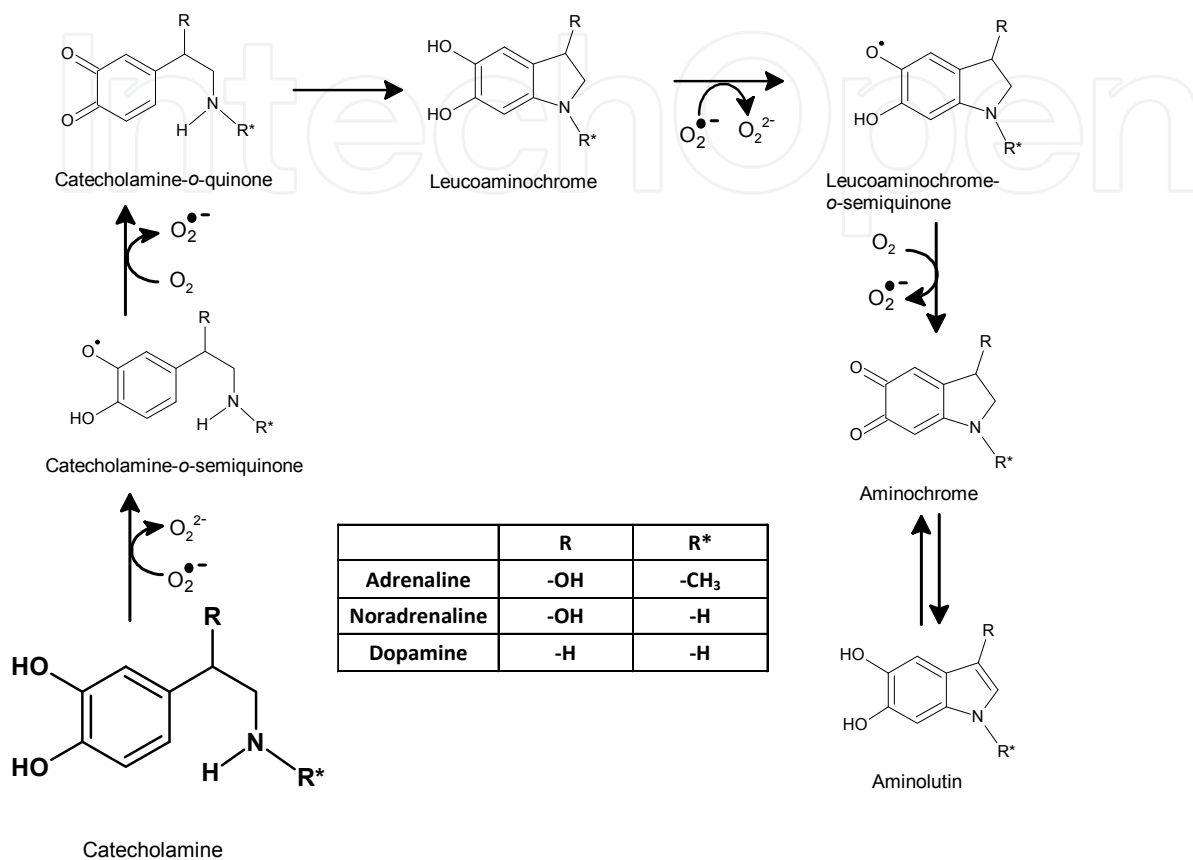


Fig. 5. Postulated pathway for the oxidation of catecholamines. The oxidation process of catecholamines initially involves their conversion to *o*-quinones through *o*-semiquinones intermediates. The *o*-semiquinone reduces oxygen resulting in superoxide anion ($O_2^{\bullet-}$). The *o*-quinone can undergo an irreversible 1, 4-intramolecular cyclization, forming leucoaminochrome. The formation of aminochrome from leucoaminochrome is a reaction where a total of two electrons are removed and leucoaminochrome semiquinone is the intermediary with $O_2^{\bullet-}$ as a by-product. Aminochrome formed can lead to the formation of aminolutins.

Adrenochrome is the most studied aminochrome due to its stability (Heacock, 1959, Rupp et al., 1994). When adrenochrome is formed and ADR still exists in solution, adrenochrome accelerates the oxidation process of the remaining ADR (Bindoli et al., 1999, Costa et al., 2007). Furthermore, the yield of adrenochrome increases if ADR semiquinone reacts with O_2 to form $O_2^{\bullet-}$ (Costa et al., 2007). This seems to be a general phenomenon of aminochromes, since it occurs also with the other catecholamines (Heacock, 1959, Bindoli et al., 1992).

Once formed, the aminochromes can be transformed into melanins, since they are reactive compounds that easily undergo a series of reactions among them. *In vivo*, this oxidation pathway may be more complex, since other factors such as metal ions or other nucleophilic

groups can be involved (Spencer et al., 1998, Bindoli et al., 1999, Spencer et al., 2002, Costa et al., 2007, Costa et al., 2009a).

At physiological pH, the oxidation pathway of biogenic catecholamines is similar, as they share similar intermediates, however their stability is fairly different (Rupp et al., 1994). The rate of internal cyclization determines the probability of nucleophilic attack to the quinone intermediates (Rupp et al., 1994, Spencer et al., 1995, Alhasan and Njus, 2008). Thus, if the internal cyclization rate is low, the quinone is likely to be attacked by external nucleophilic groups such as sulfhydryl (-SH), hydroxyl (-OH), and amine (-NH₂) groups. The slowest cyclization rate is observed for dopamine, while ADR has the higher cyclization rate, 140 times higher than NA. Thus, the probability of external nucleophilic attack to occur is as following: dopamine > NA > ADR (Rupp et al., 1994). In fact, dopamine oxidized forms were found bound to proteins, as well as to other nucleophilic molecules, as cysteine or glutathione (Fornstedt et al., 1990, Patel et al., 1991, Segura-Aguilar et al., 1997, Byington, 1998, Spencer et al., 1998, Spencer et al., 2002, Miyazaki et al., 2006). Pathognomonic significance has been given to these products, namely in Parkinson's disease (Spencer et al., 1995, Shen et al., 1996, Spencer et al., 1998). As stated, the fastest cyclization rate occurs in ADR (Bindoli et al., 1992); however, GSH adducts and quinoproteins of ADR have been found in cells, thus showing that ADR quinone can also undergo attack from cellular nucleophiles (Costa et al., 2007, Costa et al., 2009a, Costa et al., 2009c). These changes can ultimately cause cellular injury (Costa et al., 2011).

10. Adrenergic receptors

10.1 Historic introduction and background

More than one hundred years ago, Langley proposed, for the first time, the idea of transmitter receptors, by stating "(...) neither the poisons nor the nervous impulse act directly on the contractile substance of the muscle but on some accessory substance. Since this accessory substance is the recipient of stimuli which it transfers to the contractile material, we may speak of it as the receptive substance (...)" (Langley, 1905).

The receptors for NA and ADR are nowadays called adrenoceptors. The adrenoceptors are the cell membrane sites where NA and ADR act. Several discoveries were performed before the concept of adrenoceptors was fully accepted. Dale in 1905 verified that the pressor effect of ADR was reversed by ergotoxine into a depressor effect. Later on, Barger and Dale verified that ADR when injected was able to dilate some vascular beds while constricting others (Barger and Dale, 1910). These facts were ignored until 1948 when Ahlquist performed a series of experiments with several sympathomimetic amines. He concluded that the variation in the pharmacological responses of several sympathomimetic agonists in different organs was related to the different types of receptors involved (Ahlquist, 1948). To the first type of adrenoceptors, he called α and to the second β (Ahlquist, 1948). The scientific contemporary community was not prompt to accept this idea and the theory of two different "sympathins" remained (Cannon and Rosenblueth, 1933). More than ten years later other works confirmed the theory by Ahlquist through the identification of selective antagonists for the two receptor families: phentolamine and ergotamine for α -adrenoceptors; dichloroisoprenaline (Powell et al., 1958) and propranolol for β -adrenoceptors (Black et al., 1964).

The development of more selective drugs and the use of molecular cloning technology showed that several adrenoceptor subtypes exist. As Ahlquist stated, two main classes are known, α - and β -adrenoceptors. Each group is further subdivided so that five subtypes are presently recognized: two main α -receptor subtypes (α_1 and α_2) and three β -receptor subtypes (β_1 , β_2 and β_3) (Alexander et al., 2008). The α -receptor subtypes are nowadays accepted to be six subtypes in total (α_{1A} , α_{1B} , α_{1D} , $\alpha_{2A/D}$, α_{2B} , α_{2C}). Other two adrenoceptor candidates were described (α_{1L} and β_4), that may be conformational states of α_{1A} and β_1 -adrenoceptors, respectively (Guimarães and Moura, 2001, Alexander et al., 2008).

The pharmacological actions of NA and ADR have been extensively compared *in vivo* and *in vitro*. Both drugs are direct agonists on effectors cells and their actions differ mainly in their ability to stimulate α - and β -receptors. ADR and NA are approximately equipotent in stimulating β_1 -receptors. NA is a potent α -agonist and has relatively little action on β_1 -receptors; however, NA is somewhat less potent than ADR on α receptors in most organs (Westfall and Westfall, 2006). The rank order of potency is isoproterenol > ADR \geq NA for β -adrenergic receptors and ADR \geq NA \gg isoproterenol for α -adrenergic receptors (Guimarães, 1975, Westfall and Westfall, 2006, Rang et al., 2007).

In the present review, the classical pharmacological point of view will be approached as it was historically the starting point in the study of the neurotransmission of catecholamines. Although not excluding neurotransmission plasticity and adaptation as key factors for the full understanding of the topic, these issues are still under debate, so they will not be addressed here.

The knowledge of the characteristics of adrenergic receptors and the biochemical and physiological pathways that they regulate increased the understanding of the seemingly contradictory and variable effects of catecholamines on different organs. Although structurally related, different receptors regulate distinct physiological processes by controlling the synthesis or release of a variety of second messengers. The adrenoceptors are coupled to second-messenger systems via G proteins. The responses that follow the activation of adrenergic receptors result from G protein-mediated effects with the generation of second messengers and/or the activation of ion channels (Guimarães and Moura, 2001) (Figure 3).

10.2 Alpha (α)-adrenoceptors

α_1 -Adrenoceptors are found in the smooth muscle of the blood vessels, bronchi, gastrointestinal tract, uterus, and bladder. The activation of these receptors is mainly excitatory and results in the contraction of smooth muscle. However, the smooth muscle of the gut wall (but not that of the sphincters) becomes relaxed after activation of α_1 -receptors. Overall, the α_1 -adrenoceptors cause vasoconstriction, relaxation of gastrointestinal smooth muscle, salivary secretion, and hepatic glycogenolysis (Pocock and Richards, 2006, Rang et al., 2007). α_1 -Adrenoceptors are mainly coupled to $G_{q/11}$ -protein with consequent activation of phospholipase C. Phospholipase C promotes the hydrolysis of phosphatidylinositol bisphosphate producing inositol trisphosphate and diacylglycerol (Docherty, 1998, Zhong and Minneman, 1999, García-Sáinz et al., 2000, Guimarães and Moura, 2001), which act as second messengers. Inositol trisphosphate mediates intracellular Ca^{2+} release from non mitochondrial pools and, consequently, the activation of other Ca^{2+} and calmodulin

sensitive pathways such as CaMKII, whilst diacylglycerol activates PKC (Guimarães and Moura, 2001, Westfall and Westfall, 2006). The three cloned α_1 -adrenoceptor subtypes have significant differences in G protein coupling efficiency ($\alpha_{1A} > \alpha_{1B} > \alpha_{1D}$) (Docherty, 1998, Zhong and Minneman, 1999, Guimarães and Moura, 2001).

Other signalling pathways are also activated by α_1 -adrenoceptors, namely: stimulation of phospholipase A₂ leading to the release of free arachidonate, which is degraded by cyclooxygenase and lipoxygenase to form the bioactive prostaglandins and leukotrienes; Ca²⁺ influx via protein G; and phospholipase D activation (Docherty, 1998, Zhong and Minneman, 1999, Guimarães and Moura, 2001, Westfall and Westfall, 2006). Some of the responses induced by α_1 -adrenoceptors are independent of Ca²⁺ and PKC but involve small G proteins and tyrosine kinases (Zhong and Minneman, 1999). Furthermore, α_1 -adrenoceptors are able to activate mitogen-activated protein kinase pathways in many cells (Della Rocca et al., 1997). The mitogen-activated protein kinase superfamily, which consists of extracellular signal-regulated kinases 1/2 and three stress-responsive subfamilies, the c-Jun NH₂-terminal kinases, p38-mitogen-activated protein kinase (p38-MAPK), and extracellular signal-regulated kinases, is normally stimulated by growth factors and cellular stress or inflammatory cytokines (Zhang et al., 2005).

α_2 -Adrenoceptors when located presynaptically are responsible for the inhibition of transmitter release (including NA and acetylcholine from autonomic nerves) and are considered modulators of neurotransmission (autoreceptors). The different α_2 -receptors couple to a variety of effectors (Aantaa et al., 1995, Guimarães and Moura, 2001) and share about 50% in amino acid sequence in important domains (Aantaa et al., 1995). The importance of these receptors was well demonstrated in knockout mice for α_2 -adrenoceptors (Vieira-Coelho et al., 2009). Brain tissue levels of L-3,4-dihydroxyphenylalanine, dopamine, and NA were significantly higher in the knockout mice for α_{2A} - and α_{2C} -adrenoceptors when compared to wild type. The activity of COMT was higher in all three knockout (α_{2A} -, α_{2B} - and α_{2C} -adrenoceptors), while the activities of MAO, dopamine β -hydroxylase or tyrosine hydroxylase were unchanged (Vieira-Coelho et al., 2009).

α_2 -Adrenoceptors are predominantly coupled to the inhibitory G proteins, G_i and G_(o), inhibiting the activity of adenylyl cyclase (Rouot et al., 1987, Cotecchia et al., 1990, Surprenant et al., 1992, Aantaa et al., 1995, Wise et al., 1997). The α_2 -adrenoceptor stimulation leads to the activation of Na⁺/H⁺ exchange (Limbird, 1988), inhibition of the opening of voltage-gated Ca²⁺ channels (Cotecchia et al., 1990) or activation of K⁺ channels (Surprenant et al., 1992), all resulting in membrane hyperpolarization.

α_2 -Adrenoceptors are also found at postjunctional or nonjunctional sites in several tissues, thus their activation may also lead to the activation of other intracellular pathways. In peripheral tissues, postsynaptic α_2 -receptors are found in vascular and other smooth muscle cells, in adipocytes, and in many types of secretory epithelial cells (intestinal, renal, and endocrine). The activation of α_2 -adrenoceptors causes platelet aggregation, contraction of vascular smooth muscle, and inhibition of insulin release (Aantaa et al., 1995, Pocock and Richards, 2006, Rang et al., 2007). Intracellular pathways of postjunctional α_2 -receptors are varied, with activation of phospholipase A₂, C, and D, with arachidonic acid mobilization, increase in phosphoinositide hydrolysis, and increase in the intracellular availability of Ca²⁺ (Limbird, 1988, Cotecchia et al., 1990, MacNulty et al., 1992, Kukkonen et al., 1998). In

addition, α_2 -adrenoceptors can activate mitogen-activated protein kinases (Della Rocca et al., 1997, Richman and Regan, 1998).

10.3 Beta (β)-adrenoceptors

The three β -adrenoceptor subtypes are encoded by three different genes located in human chromosomes 10 (β_1), 5 (β_2), and 8 (β_3). Approximately 60% of the amino acid sequence in membrane-spanning domains is the ligand-binding pocket for ADR and NA (Kobilka et al., 1987, Emorine et al., 1989, Guimarães and Moura, 2001).

β_1 -Adrenoceptors are found in the heart where their activation results in increased rate and force of contraction. They are also present in the sphincter muscle of the gut where their activation leads to relaxation (Pocock and Richards, 2006, Westfall and Westfall, 2006, Rang et al., 2007).

The activation of β_2 -adrenoceptors in the smooth muscle of certain blood vessels leads to vasodilatation. They are also present in the bronchial smooth muscle where they mediate bronchodilatation. Relaxation of visceral smooth muscle, hepatic glycogenolysis, and muscle tremor occur after β_2 -adrenoceptors activation. Postsynaptic β_2 -receptors can be found in the myocardium, as well as on vascular and some smooth muscle cells (Gauthier et al., 1996, Gauthier et al., 2000, Pocock and Richards, 2006, Rang et al., 2007). The stimulation of prejunctional β_2 -adrenoceptors leads to NA release in neurons (Boehm and Kubista, 2002).

β_3 -Adrenoceptors are present in adipose tissue, where they initiate lipolysis in white adipose tissue (Pocock and Richards, 2006, Rang et al., 2007). They are involved in the thermogenesis process that takes place in brown adipose tissue (Gauthier et al., 1996). β_3 -Adrenoceptors are also present in the heart although their functions are not fully understood (Gauthier et al., 1996, Gauthier et al., 2000).

All β -receptor subtypes (β_1 , β_2 , and β_3) are coupled to the stimulatory G protein (G_s) leading to the activation of adenylyl cyclase and accumulation of the second messenger, cyclic adenosine monophosphate (cAMP) (Frielle et al., 1987, Emorine et al., 1989, Brown, 1990, Westfall and Westfall, 2006). Accumulation of cAMP leads to PKA activation with phosphorylation of several proteins, whose functions are changed as a result.

Curiously, several works indicate that, under certain circumstances, β -adrenoceptors can couple to G_i in addition to G_s . In fact, β_3 -receptors interact with both G_s and G_i , whereas β_1 -receptors couple predominantly to G_s (Asano et al., 1984, Chaudhry et al., 1994, Gauthier et al., 1996). β_2 -Receptors usually bind to G_s but, after sustained activation, they couple to G_i (Xiao et al., 1995, Lefkowitz et al., 2002).

10.4 Location and regulation of adrenoceptors

The adrenoceptors are targets for many important drugs in therapeutics, including those used for treatment of cardiovascular diseases, asthma, prostatic hypertrophy, nasal congestion, obesity, and pain (Guimarães and Moura, 2001, Pocock and Richards, 2006). From a therapeutic standpoint, there are many occasions where the β -adrenoceptor selective stimulation (asthma, atrioventricular block, obesity) or blockage (hypertension, coronary insufficiency) is desired. α_2 -Agonists are used in hypertension and α_1 -agonist in

nasal congestion (Aantaa et al., 1995, Guimarães and Moura, 2001, Westfall and Westfall, 2006, Rang et al., 2007). Inhibition of α_1 -adrenoceptors has also proven useful to treat hypertension and prostate hypertrophy (Zhong and Minneman, 1999, Westfall and Westfall, 2006).

The α - and β -receptors appear to be located pre- and postsynaptically. The α_1 - and β_1 -receptors are mainly in the vicinity of sympathetic adrenergic nerve terminals in peripheral target organs, or distributed in the mammalian brain (Westfall and Westfall, 2006). The α_2 - and β_2 -receptors are heterogeneously distributed. Both α_2 - and β_2 -receptors may be situated at sites (vascular smooth muscle, platelets and leukocytes) that are relatively remote from nerve terminals and may be activated preferentially by circulating catecholamines (in particular ADR) (Westfall and Westfall, 2006). On the other hand, presynaptically located α_2 - and β_2 -adrenoceptors have important roles in the modulation of neurotransmitter release from sympathetic nerve endings (Figure 3).

Responses mediated by adrenoceptors are not static, as they can be modulated and adjusted. The number and function of adrenoceptors on the cell's surface and their elicited responses may be altered by catecholamines themselves, by other hormones and drugs but they can also change with age and disease. The release of neurotransmitters can be modulated by prejunctional autoreceptors. NA or ADR, neuropeptide Y, and ATP (the last two are frequent cotransmitters in the adrenergic transmission) elicit a feedback response on prejunctional receptors (Boehm and Kubista, 2002, Westfall et al., 2002). The most studied autoreceptors have been the prejunctional α_2 -adrenergic receptors (Aantaa et al., 1995). The α_{2A} - (α_{2D} -, depending on the species investigated) and α_{2C} -adrenergic receptors are the principal prejunctional receptors that inhibit sympathetic neurotransmitter release, whereas the α_{2B} -adrenergic receptors may also inhibit the release of transmitters at selected sites (Boehm and Kubista, 2002). Antagonists of these receptors, in turn, can enhance the electrically evoked release of sympathetic neurotransmitters. Neuropeptide Y, acting on Y2 receptors (Chen et al., 1997), and ATP-derived adenosine, acting on P1 (A1) receptors, can also inhibit sympathetic neurotransmitter release, while nucleotides, acting on P2 receptors have inhibitory or facilitator effects, depending on the tissue evaluated (Driessen et al., 1994, Boehm and Kubista, 2002, Hoffmann, 2004). Other heteroreceptors present in sympathetic nerve varicosities can likewise inhibit the release of sympathetic neurotransmitters, namely through the activation of M_2 and M_4 muscarinic, 5-HT, prostaglandin E_2 , histamine, enkephalin, and dopamine receptors (Lefkowitz et al., 1990, Gainetdinov et al., 2004, Hata et al., 2004).

Enhancement of sympathetic neurotransmitter release can occur after the activation of presynaptic β_2 -adrenergic, angiotensin II, and nicotinic receptors. All of these receptors can be targets for agonists and antagonists (Boehm and Kubista, 2002).

The modulation of synaptic transmission is a crucial step in homeostasis, where receptors have key roles. The continuous exposure to adrenergic agonists causes a progressive decrease in the capacity to respond to those agents by catecholamine-sensitive cells. This phenomenon, often termed refractoriness, or desensitization, is another "check point" in neuroendocrine regulation (García-Sáinz et al., 2000, Hoffmann, 2004). Two major types of desensitization have been distinguished: homologous and heterologous. In the homologous, reduced responsiveness is observed exclusively in the receptor originally stimulated. In heterologous desensitization, a decreased responsiveness is observed to an agent or agents

unrelated to the initial stimulus. Certainly, this classification is only operational and both types of desensitization may occur simultaneously in the cells (García-Sáinz et al., 2000). Even so, the mechanisms are equally complex and need further characterization.

β -Adrenoceptor feedback regulation is common; however, β -receptors differ in the extent to which they can undergo such regulation, with the β_2 -receptors being the most susceptible. Upon challenge with an agonist, the β_2 -receptor couples to G_s and activates adenylyl cyclase to form cAMP. cAMP leads to stimulation of cyclic AMP-dependent protein kinases, like PKA, with consequent phosphorylation of β -receptor. That phosphorylation provides the signal for β -arrestin recruitment. Arrestins constitute a large family of widely expressed proteins (Baillie and Houslay, 2005, Westfall and Westfall, 2006). β -arrestin translocation from the cytosol to the activated β -receptor physically blocks the interaction of the receptor with its cellular effectors, presumably due to steric hindrance (Lefkowitz and Shenoy, 2005). The receptor phosphorylation followed by β -arrestin binding has been linked to subsequent endocytosis of the receptor. The capacity of β -arrestins to bind to the structural protein clathrin, initiating the internalization of phosphorylated receptors into vesicles, facilitates endocytosis (Goodman et al., 1996, Nelson et al., 2008). In addition to blunting responses that require the presence of the receptor on the surface of the cell, these regulatory processes may also contribute to novel mechanisms of receptor signalling via intracellular pathways (Baillie and Houslay, 2005).

Receptor desensitization may also be mediated by second messenger feedback. For example, β -adrenoceptor-mediated cAMP accumulation leads to activation of PKA, which can phosphorylate residues of β -receptors, which results in their own inhibition. For the β_2 -receptor, phosphorylation occurs on serine residues both in the third cytoplasmic loop and in the carboxyl terminal tail of the receptor. β_3 -Receptors do not suffer down regulation, since they lack recognition sites for the cAMP dependent kinases activated by stimulation of β -adrenoceptors (Gauthier et al., 1996).

Although less addressed, α -receptors desensitization has gained increased interest (García-Sáinz et al., 2000). Current data indicate that the decrease in receptor activity is associated with a homologous desensitization mechanism. The activation of PKC by Gq-coupled receptors may lead to phosphorylation of this class of G protein-coupled receptors. That phosphorylation constitutes a very substantial sterical impediment for the effective interaction of receptors with G proteins. Receptor phosphorylation is associated with receptor internalization and β -arrestins are involved (García-Sáinz et al., 2000).

In the case of α -receptors heterologous desensitization, it is not completely clear whether kinases, arrestins, or other molecules play the main role (García-Sáinz et al., 2000). However, activated PKA or PKC may phosphorylate any structurally similar receptor with the appropriate consensus sites for phosphorylation, a process which is considered a heterologous desensitization (Gainetdinov et al., 2004, Hoffmann, 2004).11. Summary

The autonomic nervous system is considered responsible for organ specific adjustments to the environment, while the endocrine system regulates more generalized adaptations by releasing hormones into the systemic circulation that act on “distant places” (Westfall and Westfall, 2006). NA and ADR play crucial roles in the interaction between the autonomic and endocrine systems when the body has to adjust to several and varied situations. It is of utmost importance that their roles and all the participants in the “adrenergic” system are

well known. It is absolutely certain that, as it happened during the twentieth century, the new century will bring new and fascinating discoveries in this area that will challenge existing preconceptions. However, the knowledge gathered until this point resulted of the work of several notorious scientists whose work should not be forgotten but remembered and valued.

11. Abbreviations

Adrenaline; Epinephrine (ADR); Adenosine-5'-triphosphate (ATP); Adenosine triphosphatase (ATPase); Calcium ion (Ca^{2+}); Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII); Cyclic adenosine monophosphate (cAMP); Catechol-O-methyltransferase (COMT); Dihydroxyphenylglycol (DHPG); Deoxyribonucleic acid (DNA); Inhibitory G protein (G_i); Stimulatory G protein (G_s); 5-Hydroxytryptamine; Serotonin (5-HT); Homovanillic acid (HVA); Potassium ion (K^+); Half-saturation constant; Michaelis constant (K_m); Monoamine oxidase (MAO); Magnesium ion (Mg^{2+}); Sodium ion (Na^+); Noradrenaline; Norepinephrine (NA); Noradrenaline transporter (NET); Superoxide anion ($\text{O}_2^{\bullet-}$); Organic cation transporters (OCT); Hydroxyl radical (HO^\bullet); Phenylethanolamine N-methyltransferase; Noradrenaline N-methyltransferase (PMNT); Ribonucleic acid (RNA); Vanillylmandelic acid; Methoxy-4-hydroxymandelic acid (VMA); Vesicular monoamine transporter (VMAT).

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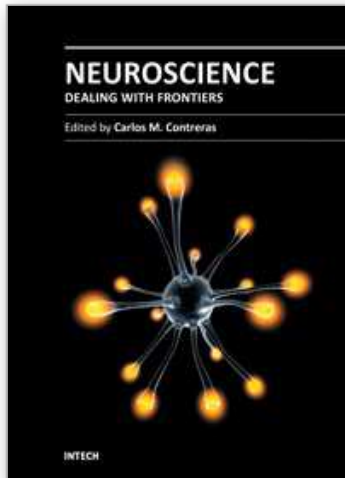
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The Neuronal Doctrine recently reached its 100th year and together with the development of psychopharmacology by the middle of 20th century promoted spectacular developments in the knowledge of the biological bases of behavior. The overwhelming amount of data accumulated, forced the division of neuroscience into several subdisciplines, but this division needs to dissolve in the 21st century and focus on specific processes that involve diverse methodological and theoretical approaches. The chapters contained in this book illustrate that neuroscience converges in the search for sound answers to several questions, including the pathways followed by cells, how individuals communicate with each other, inflammation, learning and memory, the development of drug dependence, and approaches to explaining the processes that underlie two highly incapacitating chronic degenerative illnesses.

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