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Expression and function of G-protein-coupled receptors
in the male reproductive tract

MARIA CHRISTINA W. AVELLAR, MARIA FATIMA M. LÁZARI and CATARINA S. PORTO
Setor de Endocrinologia Experimental, Departamento de Farmacologia, Universidade Federal de São Paulo
Escola Paulista de Medicina, Rua Três de Maio, 100, 4° andar, Vila Clementino, 04044-020 São Paulo, SP, Brasil

ABSTRACT
This review focuses on the expression and function of muscarinic acetylcholine receptors (mAChRs), α\textsubscript{1}-adrenoceptors and relaxin receptors in the male reproductive tract. The localization and differential expression of mAChR and α\textsubscript{1}-adrenoceptor subtypes in specific compartments of the efferent ductules, epididymis, vas deferens, seminal vesicle and prostate of various species indicate a role for these receptors in the modulation of luminal fluid composition and smooth muscle contraction, including effects on male fertility. Furthermore, the activation of mAChRs induces transactivation of the epidermal growth factor receptor (EGFR) and the Sertoli cell proliferation. The relaxin receptors are present in the testis, RXFP1 in elongated spermatids and Sertoli cells from rat, and RXFP2 in Leydig and germ cells from rat and human, suggesting a role for these receptors in the spermatogenic process. The localization of both receptors in the apical portion of epithelial cells and smooth muscle layers of the vas deferens suggests an involvement of these receptors in the contraction and regulation of secretion.

Key words: muscarinic acetylcholine receptors, α\textsubscript{1}-adrenoceptors, relaxin receptors, male reproductive tract.

INTRODUCTION
G-protein-coupled receptors (GPCRs) play different roles in autocrine and neuronal systems and are important in the physiology and pathophysiology of various organs. The activation of these receptors may affect cell proliferation, differentiation, growth and other functions in the male reproductive tract organs. This review focuses on the expression and function of muscarinic acetylcholine receptors (mAChRs), α\textsubscript{1}-adrenoceptors and relaxin receptors in the male reproductive tract. Firstly, the differential expression of mAChR subtypes (M\textsubscript{1}, M\textsubscript{2}, M\textsubscript{3}, M\textsubscript{4} and M\textsubscript{5}) in Sertoli cells, efferent ductules, epididymis, seminal vesicle and their pharmacological characteristics, intracellular signaling and possible interaction with growth factors are presented. Secondly, aspects of the genomic organization, tissue distribution in the male reproductive tract and pharmacological characteristics and function of the different α\textsubscript{1}-adrenoceptors subtypes (α\textsubscript{1A}-, α\textsubscript{1B}- and α\textsubscript{1D}-adrenoceptors) are reviewed. Lastly, the function and distribution in the male reproductive organs of the recently discovered relaxin receptors are discussed.
mAChRs preferentially couple with $G_{q/11}$, mobilize phosphoinositides to generate inositol 1,4,5-triphosphate (IP3) and 1,2-diacylglycerol (DAG) via activation of phosphoinositide-specific phospholipase C (PLC$\beta$), leading to an increase in intracellular Ca$^{2+}$ and protein kinase C (PKC) activity. M$_2$ and M$_4$ mAChRs preferentially couple with $G_{i/o}$ and inhibit elevated adenyllylcyclase activity. M$_2$ mAChRs, via G$\beta$$\gamma$, can also activate PLC$\beta$. Furthermore, mAChRs have also been shown to regulate other signaling pathways. Hence, both Go$\alpha_{q/11}$- and Go$\alpha_{i/o}$-coupled with mAChRs may exert effects through activation of small GTPases, such as Rho and Ras, and downstream effectors, such as phosphoinositide-3 kinases (PI3 kinases), non-receptor tyrosine kinases, and mitogen-activated protein kinases (MAP kinases) (for review see Lanzafame et al. 2003, van Koppen and Kaiser 2003). The latter signaling pathway seems to play a major role in the autocrine functions of mAChRs in terms of the control of cell growth or proliferation, secretion by epithelial cells, and apoptosis (for review see Eglen 2006).

The importance of mAChRs for fertility has been revealed by the demonstration that mAChR agonists impair fertility in male rats (Ban et al. 2002, Sato et al. 2005), but the underlying mechanisms that cause the male reproductive tract is essential to understand the role of the cholinergic nervous system in male fertility.

In an attempt to clarify the pathophysiology of idiopathic male infertility and to develop new methods for male contraception, researchers have focused on local regulators of intratesticular events (Huleihel and Lunenfeld 2004, Walker and Cheng 2005, Yan et al. 2007) and Sertoli cells play an important role in this process (Skinner 2005). Studies from our laboratory have shown the presence of M$_1$ to M$_5$ mAChRs (mRNA and protein) in primary culture of Sertoli cells from 15-day and 30-day old rats (Borges et al. 2001, Lucas et al. 2008). mAChRs have been localized in plasma membrane, cytoplasmic organelles, perinuclear region and nuclei in cultured Sertoli cells from 15-day old rats by using immunofluorescence studies and confocal laser scanning microscopy. M$_1$, M$_3$ and M$_5$ mAChR subtypes are mainly localized in the cell nuclei, with a weak to moderate staining visible in the plasma membrane and perinuclear region of Sertoli cells. On the other hand, M$_2$ and M$_4$ mAChR subtypes are predominantly localized in the plasma membrane, with some staining in the perinuclear region (Lucas et al. 2008). Although the functional significance of mAChRs in the plasma membrane is well established, their presence in subcellular regions has been observed in several cell types (Bernard et al. 1999, 2003, Salamanca et al. 2005). The number of receptors at the plasma membrane is frequently regulated to modulate cell responses upon stimulation. Therefore, the intracellular pool of mAChRs may represent both newly synthesized and recycled stores of receptors in amounts that vary with the balance between the rate of synthesis and the shedding and desensitization processes. The neurochemical environment may contribute to the control of the abundance and availability of cell surface receptors and, consequently, to the control of neuronal sensitivity to neurotransmitters or mAChR agonists, by regulating their delivery from the endoplasmic reticulum and Golgi complex (Bernard et al. 1999, 2003).

The presence of mAChRs gives support to the idea that the cholinergic neurotransmission may have a physi-
cultured Sertoli cells from immature hamster (Davenport and Heindel 1987) and induces phosphoinositide hydrolysis and time-dependent stimulation of the activator protein-1 DNA-binding activity in cultured Sertoli cells from 30-day old rats (Borges et al. 2001). The activation of mACHRs also rapidly stimulates [methyl-\(^3\)H]thymidine incorporation in cultured Sertoli cells obtained from 8-day and 15-day old rats, in a concentration-dependent and time-dependent manner, suggesting an induction of DNA synthesis and/or DNA repair (Lucas et al. 2004). Furthermore, the activation of mACHRs induces transactivation of the epidermal growth factor receptor (EGFR) through \(\beta\gamma\)-subunits of G proteins that promote Src-mediated metalloprotease-dependent cleavage and release of EGFR ligands from the cell surface and binding to EGFR and activation of extracellular signal-regulated kinases (Erk)1/2 in Sertoli cells from 15-day old rats. PLC\(\beta\) and intracellular Ca\(^{2+}\) mobilization, but not PKC, are involved in the Erk1/2 phosphorylation induced by mACHRs in Sertoli cells (Lucas et al. 2008). The transactivation of EGFR by the agonist-mACHR complex is also involved in the Sertoli cell proliferation (Lucas et al. 2008). The characterization of the signaling pathways regulated by the agonist-mACHR complex in Sertoli cells is an important step to understand how this complex may support spermatogenesis. It is important that future studies focus on components of the MAP kinase pathway, such as transcription factors, regulatory kinases and phosphatases, and the involvement of other signaling pathways that could be mediated by mACHRs, such as activation of PI3 kinase and phospholipase A\(_2\) (PLA\(_2\)), as they may represent additional mechanisms for the regulation of protein secretion and cell junction dynamics in the testis.

In the rat epididymis, studies from our laboratory have shown the presence of mRNA for M\(_1\), M\(_2\) and M\(_3\) mACHRs in the caput and cauda region (Maróstica et al. 2001, 2005). The levels of mRNA for M\(_2\) subtype are higher in the cauda than in the caput of the epididymis. Low levels of M\(_1\) mRNA are present in the caput and cauda of the epididymis, and low levels of M\(_3\) mRNA are found in the caput region (Maróstica et al. 2005). Immunolocalization of mACHR subtypes in different ductules and epididymis has indicated a wide degree of immunostaining for each mACHR subtype in a cell-type and tissue-specific pattern (Siu et al. 2004). M\(_1\) mACHR is detected in the epithelium of the efferent ductules, while M\(_2\) and M\(_3\) mACHRs are observed in the apical region of the ciliated cells of the efferent ductules. Apical and narrow cells of the initial segment of the epididymis show a distinct staining for M\(_1\) receptor, whereas supranuclear localization is noted in principal cells of the caput of the epididymis. In addition, staining for M\(_1\) and M\(_2\) mACHRs is visible in the membrane of some epithelial cells of the caudal epididymis (Siu et al. 2006). These findings suggest a relationship between mACHRs activation and secretory processes. In fact, the activation of mACHRs stimulated chloride secretion in cultured rat epididymal epithelium (Du et al. 2006), and increases \(^{[35]}\)S-Methionine incorporation in proteins secreted by rat efferent ductules and in secreted and tissular proteins of the caput epididymis. These effects were abolished by atropine, a nonselective mACHR antagonist (E.R. Siu et al., unpublished data). Immunoreactivity for M\(_2\) mACHR is detected in the peritubular smooth muscle of the efferent ductules and along the different epididymal regions, with a strong reaction in the proximal and distal epididymis, suggesting that this receptor subtype may play a role in smooth muscle contraction (Siu et al. 2006). Penta-hexahydro-sila-difenidol (HHSiD), a M\(_2\)/M\(_3\) selective mACHR antagonist, is able to abolish contractions induced by carbachol, a stable analog of ACh, indicating the involvement of M\(_3\) mACHRs in the epididymal muscle contraction (Siu et al. 2006). Cell-specific expression of the mACHR subtypes in the efferent ductules and epididymis suggests the role of these receptors in the regulation of smooth muscle contraction and the composition of the luminal fluid, which is essential for post-testicular maturation of spermatozoa.

In the dog vas deferens, binding studies have revealed the presence of mACHRs. The prostatic region expressed a greater amount of mACHRs than the interstitium and epididymal regions (Kondo et al. 1994). mACHR is present in the epithelial cells of the ductuli efferentes, suggesting a role in the regulation of smooth muscle contraction and fluid secretion. The activation of M\(_1\) mACHRs leads to the release of ACh, which in turn activates a nonselective mACHR to induce smooth muscle contraction (Siu et al. 2004). This complex mechanism suggests a role for mACHRs in the regulation of sperm transport and motility.
potentiates $P_{2X}$ receptor-mediated contractions, but not
adrenoceptor-mediated contractions (Iram and Hoyle
2005). In rabbit vas deferens, prejunctional $M_1$ mAChRs
inhibit the release of endogenous noradrenaline (Grimm
et al. 1994) and the contractile response of vas deferens
(Shannon et al. 1993). The presence of mRNA or pro-
tein for $M_1$, $M_2$ and $M_3$ mAChRs has also been shown
in rat vas deferens (Miranda et al. 1994, 1995), and $M_1$
and $M_2$ mAChRs are involved in the contraction of the
epididymal region of rat vas deferens (Doggrell 1986).
The cell- and region-specific expression of mAChR sub-
types in vas deferens and the role of each subtype in
different species remain to be explored.

The presence of mRNA for the five mAChRs has
been shown in the rat seminal vesicle (Hamamura et al.
2006). $M_1$ mAChR is predominantly involved in the
contraction of the seminal vesicle in guinea-pigs (Eglen
et al. 1992) and rats (Hamamura et al. 2006). Immuno-
histochemical studies revealed the presence of $M_2$
and $M_3$ mAChRs in the smooth muscle layers of the rat
seminal vesicle (Hamamura et al. 2006). $M_2$ and $M_3$
mAChRs may also be involved in protein secretion since
they are present in epithelial cells of the rat seminal vesi-
cle (Hamamura et al. 2006). Other evidence that sup-
ports this suggestion is that mAChR agonists stimulate
protein secretion in cultured epithelial cells of the rat
seminal vesicle (Kinghorn et al. 1987), alkaline phos-
phatase secretion and phospholipid synthesis in the
guinea-pig seminal vesicle (Lockwood and Williams-
Ashman 1971), and fructose release from epithelial cells
of guinea-pig seminal vesicle via nitric oxide produc-
tion (Ehrén et al. 1997). Apart from the effects on pro-
tein secretion, mAChRs are also involved in mitogenic
effects in cultured epithelial cells from the rat seminal
vesicle (Kinghorn et al. 1987). Recent studies from our
laboratory have shown that the mechanism by which
agonist-mAChR acts in seminal vesicle involves the
transactivation of the EGFR through $G\beta\gamma$-subunits pro-
teins that promote Src-mediated metalloprotease-de-
pendent cleavage and release of EGFR ligands from
the cell surface and binding to EGFR and Erk1/2 activation.
Furthermore, PLC$_i$, intracellular Ca$^{2+}$ mobilization and
et al., unpublished data). Further studies are necessary
to understand the role of the agonist-mAChR-Erk1/2 in
the proliferation, differentiation and secretion in the sem-
inal vesicle and, consequently, the impact of the cholin-
ergic nervous system to male fertility.

The presence of $M_2$ mAChR in the rat prostate
has been shown by immunoprecipitation studies (Rug-
gieri et al. 1995). On the other hand, binding studies
with selective antagonists suggested the presence of $M_3$
mAChRs (Lau and Pennefather 1998), and immunostain-
ing confirmed the presence of $M_1$ mAChR in the outer
muscle layer surrounding the prostatic acini (Nadelhaft
2003). In fact, mAChR agonist-induced contractions of
the ventral lobe of the rat prostate seem to be mediated
by $M_1$ mAChR (Lau and Pennefather 1998, Ventura et
al. 2002). The expression of mAChR subtypes varies
among the different lobes of the rat prostate; immuno-
histochemistry combined with RT-PCR analysis suggests
that $M_3$ mAChRs are predominantly expressed in the
ventral lobe and $M_2$ mAChRs in the dorsolateral lobe
(Pontari et al. 1998). The activation of mAChRs in rat
prostate gland induces glandular secretion (Wang et al.
1991) and increases Ca$^{2+}$ with concomitant activation
of $K^+$ and Cl$^-$ channels, which are essential for fluid secre-
tion (Kim et al. 2005).

$M_2$ mAChR mRNA (Obara et al. 2000) and pro-
tein (Yazawa et al. 1994) are detected in cultured pro-
state smooth muscle cells from human patients with be-
jign prostatic hypertrophy. Additionally, $M_1$ mAChRs
are present on glandular epithelial cells from these
patients (Ruggieri et al. 1995) and $M_1$ mAChR is pres-
ent in the LNCaP prostatic cancer cell line (Rayford et
al. 1997). Other studies in prostate cell lines have re-
vealed controversial findings. LNCaP cells, as well as
DU145 cells, express approximately equal amounts of
$M_1$ and $M_2$ mAChRs, whereas PC3 cells express only
$M_3$ mAChRs (Luthin et al. 1997), and mAChR agonist
increases phosphatidylinositol turnover in PC3, but not
in DU145 or LNCaP cell lines.

A high expression of $M_3$ mAChRs has been cor-
related with differentiation of the human prostatic ep-
ithelium (Blanco and Robinson 2004). Normal prostate
served in Jun N-terminal kinase activity (Ruggieri et al. 1995, Luthin et al. 1997). On the other hand, mACHR agonist induces growth of prostate epithelial cells and an increase in Erk activity in several prostate cancer cell lines (Rayford et al. 1997, Luthin et al. 1997), while in PC3 prostate carcinoma cells, mACHR activation induces transactivation of EGFR (Prenzel et al. 1999). Moreover, several studies suggest a direct or indirect role for the Erk1/2 signaling pathway in the development of prostate cancer (Papatsoris et al. 2007), but reports of Erk1/2 activation in prostate cancer have been controversial. Further characterization is necessary to understand the role of the agonist-mACHR complex and the intracellular signaling pathways involved in secretion, proliferation and differentiation in the prostate.

Multiple factors may be involved in the regulation of mACHRs. Briefly, studies have pointed out the importance of a physiological balance between androgen and testicular factors in the regulation of mACHRs in the rat epididymis (Maróstica et al. 2005). Moreover, androgen also modulates mACHRs present in the rat vas deferens (Longhurst and Brotcke 1989) and prostate (Shapiro et al. 1985).

α₁-ADRENOCEPTORS: SUBTYPES AND EXPRESSION IN THE MALE REPRODUCTIVE TRACT

The natural adrenergic amines, adrenaline and noradrenaline, are among the most important regulators of physiological and biochemical processes in the organism, through their release by adrenal medulla and related chromaffin structures and the sympathetic nervous system (for review see Docherty 2002, Jackson and Cunnane 2001, Eisenhofer 2001). With adrenergic nerve stimulation, noradrenaline is released from the nerve terminal. The action of this neurotransmitter can be mediated on the target cell by three subfamilies of G-protein-coupled receptors (α₁, α₂ and β-adrenoceptors), each of them comprising different receptor subtypes which are products of separate genes: α₁ (α₁A-, α₁B- and α₁D-adrenoceptors); α₂ (α₂A-, α₂B- and α₂C-adrenoceptors) and β (β₁-, β₂- and β₃-adrenoceptors). They play important roles in the regulation of diverse physiological functions, including cardiovascular, respiratory, urogenital and mental disorders (for review see Docherty 2002, Jackson and Minneman 1999, Brodde and Leineweber 2004). In this review, aspects of the genomic organization, structure, tissue distribution in the male reproductive tract and function of α₁-adrenoceptors are presented.

α₁-adrenoceptor subtypes (α₁A-, α₁B- and α₁D-adrenoceptor) are encoded by three separate genes (noted ADRA1A, ADRA1B and ADRA1D) as single protein products (Morrow and Creese 1986, Schwarting et al. 1990, Ramarao et al. 1992, Yang-Feng et al. 1994, Hieble et al. 1995, Piascik et al. 1995, Docherty 1998). All α₁-adrenoceptor subtypes contain:

1) a common overall structure with seven hydrophobic transmembrane (TM1-TM7) helices interconnected by hydrophilic sections composed of an intracellular and three extracellular loops;

2) an extracellular N-terminus which contains consensus sites for N-linked glycosylation (Sawutz et al. 1987);

3) an intracellular C-terminus which contains consensus sites for phosphorylation by protein kinases (Leeb-Lundberg et al. 1985, Lefkowitz 1998, Lefkowitz et al. 1998, Vázquez-Prado et al. 2000) and interaction with regulatory proteins (Hieble et al. 2001, Pupo and Minneman 2003, Diviani et al. 2003); and


All three α₁-adrenoceptor genes present a genomic structure of at least two exons separated by an intron ranging from 7.2–93 kbp in different species (Yasuoka et al. 1996, Perez et al. 1994, Ramarao et al. 1992, Yang-Feng et al. 1990). The first exon contains the nucleotide sequence codeing for the N-terminus through the transmembrane intracellular and three extracellular loops; 2) an extracellular N-termini which contains consensus sites for N-linked glycosylation (Sawutz et al. 1987);
domain TM6 of the receptor, while the second exon encodes the third extracellular loop, transmembrane domain TM7 and all (or most) of the C-terminal region (Ramarao et al. 1992, Esbenshade et al. 1995). Additional exons are known to be present in the ADRA1A gene in human (Hirasawa et al. 1995, Chang et al. 1998, Cogé et al. 1999, Patrão et al. 2008), rabbit (Suzuki et al. 2000), guinea-pig (González-Espinosa et al. 2001) and rhesus monkey (Patrão et al. 2008), whose alternative use leads to several transcript variants by splicing mechanisms. In humans, at least eleven different ADRA1A splice variants have been reported (Hawrylyshyn et al. 2004). A summary of the nomenclature of the genes, transcripts and their corresponding isoforms for all these splicing variants can be found in Patrão et al. (2008). Four of these variants, differing in length and sequence of the C-terminal region, are functional proteins with pharmacological profile and ability to mediate effects induced by norepinephrine similar to the classical α1A-adrenoceptor (wild type, ADRA1A_1) (Hirasawa et al. 1995, Chang et al. 1998, Cogé et al. 1999, Hawrylyshyn et al. 2004). They present, however, differences in G-protein coupling specificity and down-regulation mechanisms are suggested among these splice variants (Hirasawa et al. 1995, Chang et al. 1998, Price et al. 2002). The other seven human splice variants, differing in length and sequence in the C-terminal region, code for non-functional truncated isoforms that lack TM7 and, for this reason, are incapable of ligand binding and activating signal transduction (Chang et al. 1998, Cogé et al. 1999, Hawrylyshyn et al. 2004). Differences in the relative abundance, cellular localization and possible functions for these ADRA1A gene splice variants have been suggested (Hirasawa et al. 1995, Cogé et al. 1999, Schwinn and Kwatra 1998, Ramsay et al. 2004, Patrão et al. 2008). Functional and truncated ADRA1A splice variants are differentially expressed in different tissues of the male reproductive tract of humans and rhesus monkeys (testis, epididymis, prostate and seminal vesicle), raising the question about their relevance in physiological and pathological events in these tissues (Patrão et al. 2008). Splice variants in humans and other species, however, remains to be determined.

All three α1-adrenoceptor subtypes show similar potencies and intrinsic efficacies to endogenous ligands released from the sympathetic fibers (noradrenaline and adrenaline) and high affinity to the nonspecific α1-adrenoceptor antagonist prazosin (Hieble and Ruffolo 1996). A fourth subtype has been proposed based on its lower affinity to prazosin. The receptor subtype, designated α1L-adrenoceptor, is probably not a distinct receptor but rather a low-affinity state of the α1A-adrenoceptor (Ford et al. 1997, Daniels et al. 1999) and has been reported to be involved in smooth muscle contraction of human, rabbit and dog lower urinary tract tissues (Muramatsu et al. 1994, Ford et al. 1996, Testa et al. 1996, Fukasawa et al. 1998) with an important role in the treatment of stress urinary incontinence (Bishop 2007). The antagonists 5-methyl urapidil, WB 4101 and niguldipine show higher affinity for α1A-adrenoceptors, while BMY 7378 recognizes preferentially α1D-adrenoceptors (Deng et al. 1996, Saussy et al. 1996, Yang et al. 1997). The alkylation agent chloroethylclomine is an irreversible antagonist that mostly inactivates both α1B- and α1D-adrenoceptors (Laz et al. 1994, Hieble et al. 1995, Xiao and Jeffries 1998).

α1-adrenoceptors are preferentially coupled with Gq/11 protein and activate phospholipase Cβ to form DAG and IP3, inducing consequent increase in cytosolic concentration of Ca2+ and PKC, which in turn phosphorylates several substrates (Zhong and Minneman 1999, Hein and Michel 2007). The relative coupling efficiencies to second messenger formation differs depending on the receptor subtype (α1A > α1B > α1D; Theroux et al. 1996, Zhong et al. 2001). There are also evidences that, depending on the cell or tissue which were analyzed, α1-adrenoceptors can also couple with different G protein (Gi, Gs and Gq; Horie et al. 1995, Hu and Nattel 1995, Chen et al. 1996, Nakaoka et al. 1994, Shinoura et al. 2002) activating different signaling pathways to modulate cellular function (Michelotti et al. 2000, Wier and Morgan 2003) and possibly modulating L-type calcium, sodium and cation channels from
The complexity of the events involved in α₁-adrenoceptor activation is further demonstrated by the fact that several interacting proteins can allow differential signalling, regulation and subcellular localization for each subtype α₁-adrenoceptors (Hu and Nattel 1995, Chen et al. 1996, Wang et al. 2005, Xu et al. 1999). Also, there are evidences that α₁A-, α₁B- and α₁D-adrenoceptors are all capable to form homodimers and heterotrimers (Vicentic et al. 2002). Oligomerization of α₁B-adrenoceptors seems to be required for proper receptor function, since mutations of putative key hydrophobic residues in transmembrane domain TM1 and transmembrane domain TM4 prevent receptor oligomerization and result in impaired α₁B-adrenoceptor cell-surface expression and signaling (Lopez-Gimenez et al. 2007). α₁-adrenoceptors are also capable to heterodimerize (Uberti et al. 2003, 2005, Hague et al. 2004a).

The subcellular localization of the α₁-adrenoceptor subtypes can also confer distinct receptor function, as known to occur with other G-protein-coupled receptors (for review see Gobeil et al. 2006). The presence of intracellular α₁-adrenoceptors has been described in the literature (Piascik and Perez 2001, Hirasawa et al. 2001, Toews et al. 2003). Studies with recombinant receptor have identified a characteristic distribution of α₁A-adrenoceptors throughout the cytoplasm of the cell, whereas α₁B-adrenoceptors mainly present classic plasma membrane labeling pattern (Hrometz et al. 1997, Sugawara et al. 2002). Intracellular staining of α₁A-adrenoceptors in epithelial cells of the epididymis, vas deferens and seminal vesicle of rats has been also reported (Queiróz et al. 2008), confirming that this location of the receptor might be important in physiological events. In human prostatic smooth muscle cells, approximately 40% of the α₁-adrenoceptors were intracellular, particularly concentrated around the nucleus (Mackenzie et al. 2000). Similar results were observed in cultured vascular smooth muscle cells and rat-1 fibroblasts transfected with the α₁-adrenoceptor subtypes, although significant expression of α₁A-adrenoceptor in these cells was located in the plasma membrane (Hrometz et al. 1999, McCune et al. 2000). Conversely, α₁D-adrenoce
Functional studies also reveal that a predominant population of $\alpha_1\text{A}$-adrenoceptors is involved in the contractile response of smooth muscle from vas deferens, prostate and seminal vesicle (Mallard et al. 1992, Aboud et al. 1993, Yazawa and Honda 1993, Lepor et al. 1994, Teng et al. 1994, Burt et al. 1995, 1998, Pupo 1998, Silva et al. 1999). Interestingly, the presence of $\alpha_1\text{A}$- and $\alpha_1\text{B}$-adrenoceptors is detected in rat vas deferens by radioligand binding studies (Hanft and Gross 1989, Salles and Badia 1991, Vivas et al. 1997), indicating that the correlation of binding and functional assays are not always directly correlated (Zhong and Minneman 1999). However, the contractions of vas deferens from cyproterone acetate-treated rats involves the participation of both $\alpha_1\text{A}$- and $\alpha_1\text{B}$-adrenoceptors, indicating that this antiandrogen induces plasticity in the functional $\alpha_1\text{A}$-adrenoceptor subtypes in the rat vas deferens (Campos et al. 2003). Divergence between receptor detection through functional methods versus molecular biology methods has also been discussed by Forray et al. (1994) and MacKinnon et al. (1994). Participation of $\alpha_1\text{A}$-adrenoceptor in the contractile response of cauda epididymis from mouse (Hib and Caldeyro-Barcia 1974), in spontaneous contractions of rat epididymis regions (Da Silva et al. 1975, Hib 1976, Chaturapanich et al. 2002) and in events related to epithelial function such as electrolyte transport (Leung et al. 1992, Chan et al. 1994) and protein processing (Ricker et al. 1996) have been reported. A predominant population of $\alpha_1\text{A}$-adrenoceptor in rat epididymis has been also confirmed by radioligand binding assays (Queiróz et al. 2002) and immunohistochemistry, which located this receptor subtype in smooth muscle and in subpopulations of epithelial cells from human and rat epididymis (Queiróz et al. 2008) and rhesus monkey (Patrão et al. 2008). The expression of different $\alpha_1$-adrenoceptor mRNA subtypes are known to occur along rat epididymis (Queiróz et al. 2002), raising the possibility of possible differential importance of $\alpha_1$-adrenoceptor subtypes in different epididymal cells during physiological and pathological events, including effects on male fertility. In fact, studies by Bhatel et al. (1974) and Ratnasooriya and Wadsworth (1990, 1994) with different $\alpha_1$-adrenoceptor antagonists induces a decrease in ejaculatory capacity associated with a reduction in the fertilization ability of the sperm, suggesting a role for the sympathetic nervous system in fertility maintenance via $\alpha_1$-adrenoceptors (Ratnasooriya and Wadsworth 1990, 1994).

Although it is known that, in most tissues, mixtures of $\alpha_1$-adrenoceptor subtypes (mRNA and protein) are concomitantly expressed, with variable relative expression levels (Zhong and Minneman 1999), quantification and localization of all three $\alpha_1$-adrenoceptor subtypes at protein level have been difficult to be determined mainly due to the lack of pharmacological tools with selectivity or specificity, and the high degree of conservation of the $\alpha_1$-adrenoceptor subtypes. Selective antibodies against ADRA1A, ADRA1B and ADRA1D have been successfully reported in few studies. Detection of these proteins in tissue extracts by Western blot analysis has been shown with different rat tissues, including vas deferens and prostate (Manni et al. 2005, 2006, Shen et al. 2000). Immunohistochemical detection of ADRA1A in human prostate (Walden et al. 1999), human peripheral blood lymphocytes (Tayebati et al. 2000) and in different tissues of the male reproductive tract of rats, humans (Queiróz et al. 2008) and rhesus monkeys (Patrão et al. 2008) have also been reported.

GPCRS IN THE MALE REPRODUCTIVE TRACT

In addition to the influence of steroid hormones and neurotransmitters, the male reproductive tissues are also under control of other factors and hormones. Relaxin is one the novel peptide hormones which can be important for the normal male reproductive function.

Relaxin is a 6 kDa peptide that belongs to a superfamily of hormones structurally related to insulin, which also includes, among others, the insulin-like growth factors (IGFs) and the insulin-like peptide from Leydig cells (INSL3) (for review see Sherwood 2004). Relaxin was initially detected only in females as a hormone to help parturition, but it has several other functions related or not with the reproductive function (for review see Sherwood 2004). The active principle was extracted in 1930 (Fevold et al. 1930), but the purified hormone was only obtained in 1974 (for review see Sherwood 2004). Relaxin is composed of two peptide chains, A and B, which are linked by disulphide bonds. The binding site to the receptors is mainly expressed in the brain. Although the aminoacid sequences of the relaxins H1 and H2 differ considerably, their biological activity is similar, whereas the biological activity of the relaxin H3 is higher.

Only two forms of relaxin have been described in rodents. The relaxin 1 in these animals is encoded by an orthologue of the H2 gene in humans. The relaxin 1 in the mouse and rat is derived from an orthologue of the H2 gene (Bathgate et al. 2002, Burazin et al. 2002). The relaxin 1 form of relaxin so far detected in plasma from primate animals is derived from the H2 gene and its orthologue in various species.

Relaxin was discovered in 1926 by Frederic L. Hisaw, who found that the injection of serum from pregnant guinea pigs or rabbits into virgin guinea pigs produced a relaxation of the pubic ligament (Hisaw 1926). The active principle was extracted in 1930 (Fevold et al. 1930), but the purified hormone was only obtained in 1974 (for review see Sherwood 2004). Relaxin is composed of two peptide chains, A and B, which are linked by disulphide bonds. The binding site to the receptor...
tial proteolytic digestion of the signal peptide and the peptide that connects the two peptide chains (Ivell and Einspanier 2002).

Although relaxin was discovered a long time ago, the identity of its receptors remained unknown until 2002, when Hsu et al. described that relaxin could bind to and activate the G-protein-coupled receptors (GPCRs) RXFP1 and RXFP2, formerly known as LGR7 and LGR8, respectively. The human relaxins H1 and H2 and porcine relaxin 1 strongly bind to and activate both RXFP1 and RXFP2 with almost the same affinity, but the rat relaxin 1 binds only weakly to RXFP2 (Hsu et al. 2002, Scott et al. 2005). Although relaxin can bind to and activate both the recombinant RXFP1 and RXFP2 expressed in HEK 293 cells (Hsu et al. 2002), it is now recognized that RXFP1 is the endogenous receptor for relaxin, and RXFP2 the endogenous receptor for INSL3 (Zimmermann et al. 1999, Feng et al. 2006).

These two receptors belong to a subfamily of GPCRs, now called leucine-rich repeat-containing GPCRs (LGRs), which also includes the receptors for the gonadotropins LH and FSH, the TSH receptor and the more recently described sea anemone LGR (Nothacker and Grimmelikhuijzen 1993), snail LGR (Tensen et al. 1994), fruit fly LGR1 and LGR2 (Hauser et al. 1997, Nishi et al. 2000), nematode LGR (Kudo et al. 2000) and the LGRs 4, 5 and 6 (Hsu et al. 2000, 2002). Some of these LGRs (LGR 1 to 6) have still unknown ligands. Relaxin 3 binds to RXFP1, but probably its physiological target is the small peptide-like RXFP3 (Liu et al. 2003), the formerly orphan receptor GPCR 135 (for review see Bathgate et al. 2006, Halls et al. 2007).

All members of the LGR subfamily contain a large extracellular amino terminal domain consisting of three subdomains: a N-terminal cysteine-rich subdomain (NCR) followed by a leucine-rich repeat-containing subdomain (LRR subdomain) and a C-terminal cysteine-subdomain (CCR). The leucine-rich repeats in the LRR subdomain present several β-strands and have been postulated to form a horseshoe shape which is important for hormone binding (Jiang et al. 1995). The CCR subdomain is a hinge region that allows contact between the

Relaxin can increase cyclic AMP after interacting with recombinant RXFP1 and RXFP2 in heterologous systems (Hsu et al. 2002, Sudo et al. 2003, Halls et al. 2006). Relaxin can also stimulate endogenous receptors to increase cyclic AMP in many reproductive (Sanborn et al. 1980, Cheah and Sherwood 1980, Judson et al. 1980, Bradson 1978) and non-reproductive tissues (Bathgate et al. 2006), and other downstream signaling reactions can be activated after the production of cyclic AMP, such as tyrosine kinase or mitogen-activated protein kinase activation, and inhibition of phosphodiesterase (for review see Bathgate et al. 2006, Halls et al. 2007). The accumulation of cyclic AMP induced by relaxin in cells that endogenously express RXFP1 involves a time-dependent biphasic pathway: an early phase that involves coupling to Gs, and a delayed phase that involves activation of PI3K and PKCζ. In cells expressing the recombinant RXFP1, studies confirmed this biphasic pattern of cyclic AMP stimulation by relaxin, and showed that the delayed phase involving PI3K and PKCζ was dependent on βγ subunits of the Goα3. Activation of recombinant RXFP2 by INSL3, on the other hand, involved an initial activation of Goα that was modulated by an inhibition mediated by Goαb and the release of inhibitory G-βγ subunits (Halls et al. 2006). Therefore, initially both RXFP1 and RXFP2 stimulate cyclic AMP accumulation, but RXFP1 also activates a delayed pathway that further increases cyclic AMP accumulation.

With the recent availability of knockout animals for relaxin or its receptors, it has been possible to establish the physiological importance of this hormone. Relaxin has an antifibrotic effect in several tissues, and the re-

In the reproductive tract of female mice, the disruption of relaxin or Rxfp1 gene causes the same abnormalities: an absence of the relaxation and the elongation of the interpubic ligament and impaired nipple development (Zhao et al. 1999, Krajnc-Franken et al. 2004).

The physiological role of relaxin in the male is not so well defined and we will next present some data obtained in our laboratory and from the literature related to the role of relaxin in the male reproductive tissues.

The main evidence of the important role of relaxin for male reproductive function came from observations in transgenic mice with a disruption of relaxin or relaxin receptor genes. In male mice, the disruption of the relaxin gene causes a delayed development of the reproductive tract, with an arrest of sperm maturation (Samuel et al. 2003a), but the disruption of Rxfp1 does not alter testes or prostate (Kamat et al. 2004), and impair spermatogenesis and reduces fertility only in the first generations of knockout animals (Krajnc-Franken et al. 2004). The interaction of INSL3 with RXFP2 controls the differentiation of the gubernaculum, the caudal genitoinguinal ligament critical for testicular descent, and deletion of Ins3 or Rxfp2 causes cryptorchidism (Zimmermann et al. 1999). Transgenic overexpression of relaxin did not prevent cryptorchidism in Ins3 knockout animals (Feng et al. 2006). These observations strongly support the idea that, although both RXFP1 and RXFP2 can bind relaxin in vitro, relaxin does not physiologically activate RXFP2. Nevertheless, a contribution of RXFP2 to the actions of relaxin in some species cannot be completely excluded.

The relaxin source in males seems to vary depending on the species. In humans and many other mammals, relaxin is produced by the prostate and released almost exclusively in the seminal fluid. The relaxin mRNA has been detected in human prostate by RT-PCR and in situ hybridization (for review see Sherwood 2004, Ivell et al. 1989, Yki-Jarvinen et al. 1983). In other species, the testis (shark) or seminal vesicle (boar) may be the main source of the hormone (Steinetz et al. 1998, Kohsaka et al. 1992). The source of relaxin in the male rat is controversial. Immunohistochemistry studies have failed to demonstrate relaxin expression in testis, prostate, seminal vesicle and epididymis (Anderson et al. 1989). The mRNA for relaxin has been detected in prostate and testis (Gunnersen et al. 1995).

Regarding the expression of relaxin receptors in various tissues of the male tract, data available in the literature are scarce. Transcripts for both RXFP1 and RXFP2 have been identified in the whole rat testis and germ cells (Anand-Ivell et al. 2006, Filonzi et al. 2007), but in primary culture of Sertoli cells only RXFP1 transcripts have been found (Filonzi et al. 2007). RXFP2 but not RXFP1 transcripts are present in Leydig cells. The RXFP1 protein has been detected in elongated spermatids of rat Sertoli cells (Filonzi et al. 2007), and the RXFP2 protein in human Leydig and germ cells (Anand-Ivell et al. 2006). The role of relaxin receptors in the testis remains to be determined. Since RXFP1 receptors are expressed in germ cells during specific stages of the development, one may speculate that relaxin participates in the spermatogenic process. In addition, localization of RXFP1 receptors in Sertoli cells suggests that the hormone plays a role in spermatogenesis and about the role of relaxin in mature spermatozoa a controversy. Whereas some data in the literature support a role for relaxin in sperm motility (Garosi et al. 1998, Essig et al. 1982), we detected only low levels of relaxin mRNA in mature spermatozoa, and RXFP1 protein was undetectable (Filonzi et al. 2007). Other studies also failed to find an effect of relaxin on sperm function (Jockenhovel et al. 1990, Newinger et al. 1990).

The transcripts of Rxfp1 and Rxfp2 are present in the caput and cauda regions of the rat epididymis (Anand-Ivell et al. 2007). Rxfp2 transcripts were also found in human epididymis (Anand-Ivell et al. 2006). Transcripts of Rxfp1 and Rxfp2 have also been identified in the seminal vesicle, but in the prostate only Rxfp1 mRNA was detected (Filonzi et al. 2007).
Muscarinic acetylcholine receptors, $\alpha_1$-adrenoceptors and relaxin receptors are involved in the regulation of secretion. RXFP1 receptors detected in the arteriolar walls may control local vascular resistance (Filonzi et al. 2007). Furthermore, relaxin increases the expression of metalloproteinase 7 gene in the vas deferens, which may be involved in collagen and matrix remodeling and/or apoptosis (Ii et al. 2006). In fact, a significant increase in the collagen expression in the prostate and testis of relaxin knockout mice has been documented before (Samuel et al. 2003a). It remains to be investigated whether RXFP1 receptors in the epithelium of the vas deferens are involved in the regulation of secretion.

**CONCLUSIONS**

Muscarinic acetylcholine receptors, $\alpha_1$-adrenoceptors and relaxin receptors are often co-localized in tissues of the male reproductive system and may act in concert to regulate several aspects of reproduction. In testis, all subtypes of muscarinic acetylcholine receptors are found in Sertoli cells, where they might counteract FSH-induced cyclic AMP accumulation, influence protein synthesis, cell proliferation and cell junction dynamics. The relaxin receptor RXFP1 expressed in Sertoli cells increases cell proliferation and may also affect protein synthesis, acting in the same direction as FSH. These receptors may therefore indirectly affect spermatogenesis. In addition, RXFP1 is found in late stage germ cells, and muscarinic acetylcholine receptors are found in spermatocytes, supporting their role in spermatogenesis. All these GPCRs are widely distributed in the efferent ducts of the male tract: efferent ducts, epididymis and vas deferens. Their localization in epithelial cells suggests a role in secretory and absorptive processes, and they may stimulate protein synthesis, therefore affecting sperm composition.

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**RESUMO**

Esta revisão enfatiza a expressão e a função dos receptores muscarínicos, adrenoceptores $\alpha_1$ e receptores para relaxina.
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cul a seminal e próstata de várias espécies indica o envolvimento destes receptores na modulação da composição do fluido luminal e na contração do músculo liso, incluindo efeitos na fertilidade masculina. Além disso, a ativação dos receptores muscarínicos leva à transativação do receptor para o fator crescimento epidermal e proliferação das células de Sertoli. Os receptores para relaxina estão presentes no testículo, RXFP1 nas espermídias alongadas e células de Sertoli de rato e RXFP2 nas células de Leydig e germinativas de rato. A localização de ambos os receptores na porção apical das células epidérmicas e no músculo liso dos ductos deferentes de rato sugere um papel na contração e na regulação da secreção.

**Palavras-chave:** receptores muscarínicos, adrenéctores α₁, receptores para relaxina, sistema reprodutor masculino.

**REFERENCES**


**REFERENCES**


an alpha 1B-adrenoceptor mediating contraction of the rat spleen. Br J Pharmacol 115: 467–475.


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HEIN P and MICHEL MC. 2007. Signal transduction and regulation: are all alpha1-adrenergic receptor subtypes


and LGR7 and the signalling mechanism for LGR7. Mol Endocrinol 14: 1257–1271.


acetylcholine receptors: relevance to infertility and male contraception. Immun, Endoc & Metab, Agents in Med Chem 8: 42–50.


SUDO S, KUMAGAI J, NISHI S, LAYFIELD S, FERRARO T, BATHGATE RA AND HSUEH AJ. 2003. H3 relaxin is a specific ligand for LGR7 and activates the receptor by interacting with both the ectodomain and the exoloop 2. J Biol Chem 278: 7855–7862.


