REVIEW

The renin–angiotensin system and male reproduction: new functions for old hormones

P S Leung and C Sernia[1]

Department of Physiology, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong

[1]Department of Physiology and Pharmacology, University of Queensland, Brisbane, Australia

(Requests for offprints should be addressed to P S Leung; Email: psleung@cuhk.edu.hk)

Abstract

The blood-borne renin–angiotensin system (RAS) is known best for its role in the maintenance of blood pressure and electrolyte and fluid homeostasis. However, numerous tissues show intrinsic angiotensin-generating systems that cater for specific local needs through actions that add to, or differ from, the circulating RAS. The male reproductive system has several sites of intrinsic RAS activity. Recent focus on the epididymis, by our laboratories and by others, has contributed important details about the local RAS in this tissue. The RAS components have been localized morphologically and topographically; they have been shown to be responsive to androgens and to hypoxia; and angiotensin has been shown to influence tubular, and consequently, fluid secretion. Components of the RAS have also been found in the testis, vas deferens, prostate and semen. Angiotensin II receptors, type 1 and, to a lesser extent, type 2 are widespread, and angiotensin IV receptors have been localized in the prostate. The roles of the RAS in local processes at these sites are still uncertain and have yet to be fully elucidated, although there is evidence for involvement in tubular contractility, spermatogenesis, sperm maturation, capacitation, acrosomal exocytosis and fertilization. Notwithstanding this evidence for the involvement of the RAS in various important aspects of male reproduction, there has so far been a lack of clinical evidence, demonstrable by changes in fertility, for a crucial role of the RAS in male reproduction. However, it is clear that there are several potential targets for manipulating the activity of the male reproductive system by interfering with the locally generated angiotensin systems.

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Introduction

The renin–angiotensin system (RAS) is principally a blood-borne hormone system that regulates blood pressure directly by its vasopressor action and indirectly via a cluster of central and peripheral mechanisms that integrate pressor, electrolyte and fluid homeostasis (Reid *et al.* 1978, Stroth & Unger 1999, De Gasparo *et al.* 2000). The effector hormone of the RAS is predominantly angiotensin II (AngII), with AngIII, AngIV and Ang(1–7) relegated to lesser, albeit ascendant, roles. Nevertheless, the actions of the RAS reach beyond the cardiovascular system and include such diverse targets as the pancreas (recently reviewed (Leung & Carlsson 2001)), and various components of the female and male reproductive systems (Vinson *et al.* 1997, Poisner 1998, Speth *et al.* 1999). The angiotensins in these tissues are frequently of a paracrine/autocrine origin, from the activity of local tissue RAS (Deschepper *et al.* 1986, Campbell 1987, Phillips *et al.* 1993). The focus of this review is on the emerging roles for local RAS in male reproduction, with some emphasis on the contributions to our particular area of research on the expression, regulation and functions of the RAS in the epididymis.

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Table 1 Summary of the components of the RAS found in the male reproductive system. References are not exhaustive but representative. Asterisk indicates references at the gene level of RAS components; otherwise references are to the protein level of RAS components

RAS component	Location	Reference
Angiotensinogen	Testis (Leydig cells); epididymis; prostate	Dzau *et al*. 1987*, Wong & Uchendu 1991, Leung *et al*. 1999*, Pinterova *et al*. 2000*, C Sernia, unpublished observations, M E Fabiani, unpublished observations*
Renin	Leydig cells; epididymis; prostate	Pandey *et al*. 1984*a**, Deschepper *et al*. 1986*, Wong & Uchendu 1991, Pinterova *et al*. 2000*, M E Fabiani, unpublished observations
ACE	Leydig cells; seminiferous tubules; epididymis; prostate; spermatozoa; seminal plasma	El-Dorry *et al*. 1983, Pandey *et al*. 1984*b*, Berg *et al*. 1986, Wong & Uchendu 1991, Mukhopadyay *et al*. 1995, Williams *et al*. 1995*, Kohn *et al*. 1998*a*, Pinterova *et al*. 2000*, Nassis *et al*. 2001*
Angiotensin II	Testis; epididymis; prostate; seminal plasma	Okuyama *et al*. 1988, Zhao *et al*. 1996, O'Mahony *et al*. 2000, Dinh *et al*. 2001*b*
Angiotensins III, IV or (1–7)	Not reported	—
Angiotensin receptors: AT_{1a}, AT_{1b}	Testis (Leydig cells); epididymis; vas deferens; prostate; spermatozoa	Magnan & Regoli 1979, Millan & Aguilera 1988, Grove & Speth 1989, Kitami *et al*. 1992*, Sum & Cheung 1995, Vinson *et al*. 1995, Leung *et al*. 1997*b*, 1998*, Dinh *et al*. 2001*a*
Angiotensin receptor: AT_2	Testis; epididymis; prostate	Leung *et al*. 1997*b*, 1998*a**, Speth *et al*. 1999, Dinh *et al*. 2001*a*
Angiotensin binding sites: AT_4, AT (1–7)	Prostate (AT_4)	Dinh *et al*. 2001*b*

Components of the RAS in the male reproductive system

There is considerable evidence for the local synthesis of components of the RAS in male reproductive tissues, in seminal fluid and in spermatozoa (see reviews by Mukhopadyay *et al.* 1995, Vinson *et al.* 1997, Speth *et al.* 1999). AngII concentrations in the seminal plasma are three to five times higher than in blood plasma (O'Mahony *et al.* 2000). The capacity of sites in the testis, epididymis and prostate to synthesize AngII (see Table 1) and the presence of a blood–testis barrier restricting the entry of blood-borne substances, make it probable that the AngII in seminal plasma originates in the male reproductive tract. The details of male reproductive organs' ability to synthesize RAS components, at the levels of genes and/or proteins, are summarized in Table 1. Furthermore, the localization of AngII to epithelial cells in the epididymis and prostate (Zhao *et al.* 1996, Dinh *et al.* 2001*a*) suggests that this peptide is generated by both intra- and extra-cellular mechanisms. Since most AngII antisera cross-react with several angiotensins, it is also possible that other angiotensins are synthesized in addition to AngII. In this regard, the presence of angiotensin receptor subtype AT_4 in the human prostate is certainly a strong indication that the peptide AngIV is present (Dinh *et al.* 2001*b*).

The best evidence for a complete classic RAS exists for the testes, where angiotensinogen, renin, an angiotensin-converting enzyme (ACE) specific to the testis (ACET) (Esther *et al.* 1997, Hagaman *et al.* 1998), angiotensin receptor subtypes AT_1 and AT_2 and AngII have all been found. Interestingly, both renin and angiotensinogen mRNA are present and localized to the testicular Leydig cells of mouse testes (Pandey *et al.* 1984*a*, Deschepper *et al.* 1986, Dzau *et al.* 1987). These data suggest that a locally

generated RAS is operating in the testes, which may be linked to a renin-dependent biosynthetic pathway. Moreover, ACET is found exclusively in late pachytene spermatocytes and mature spermatozoa (Berg *et al.* 1986, Sibony *et al.* 1993) while the somatic form of ACE (sACE) is found in the Leydig cells, epididymis and prostate (Nassis *et al.* 2001). The importance of ACET in fertility is discussed below.

The existence of an intrinsic RAS in the epididymis has been implicated by the presence of immunoreactive AngI, AngII, renin-like activity and ACE in cultures of rat epididymis (Wong & Uchendu 1991). AngII (Zhao *et al.* 1996), AT_1 and AT_2 receptors (Grove & Speth 1989, Leung *et al.* 1997*a*) have been localized to the epididymal epithelium. Angiotensinogen, the mandatory component for locally produced angiotensins, has been identified by Northern blot, PCR and Western blot (Leung *et al.* 1999, 2000). It has been localized to the epithelium by immunohistochemistry and *in situ* hybridization histochemistry (Leung *et al.* 1999). Western blot analysis of angiotensinogen identified a single 60 kDa protein band similar to that observed for other tissues. Interestingly, renin mRNA has not been found in the epididymis (Leung *et al.* 1999, 2000), despite the presence of renin-like activity in epididymal cell cultures (Wong & Uchendu 1991). This observation implies the presence of a non-classic RAS, at least in the epididymal epithelium, that uses alternate enzymes to renin. In fact, kallikrein, a renin-like enzyme, can result in direct generation of AngII, rather than the definitive enzymatic cascade of renin and ACE arising from the classic RAS (Maruta & Arakawa 1983). In this regard, renin mRNA is also absent from rat testes (Campbell & Habener 1986, Dzau *et al.* 1987) and the carotid body (Lam & Leung 2002), indicating that renin-independence could be present in those tissues. However, it should be stressed that local angiotensins could also be generated by the action of plasma-derived renin from the kidney. While co-localization studies have not been attempted, the proximity of angiotensinogen, AngII and AT receptors suggests a paracrine/autocrine action of locally generated AngII on the secretion of anion and fluid by the epididymal epithelium function (Leung *et al.* 1997*a*). This has been found to be the case in electrophysiological studies showing the action of AngII on anion secretion to be mediated by the AT_1 receptor (Leung *et al.* 1997*b*). In addition to the epididymal epithelium, AngII receptors have also been characterized and identified in epididymal fat tissue of rat and human origin (Crandall *et al.* 1993, 1994). Recently, mRNAs for angiotensinogen, renin, ACE and AngII receptors were identified and detected in the stromal vasculature of the fat tissue (Pinterova *et al.* 2000), indicating that a complete classic RAS may be operated in rat/human adipose tissue of the epididymis. The above data support the existence of either a classic or non-classic RAS in the epididymis that may modulate, via paracrine or autocrine action, epididymal functions such as anion excretion and, consequently, sperm activity.

There is now a body of evidence for a complete classic RAS in the prostate (Dinh *et al.* 2001*a*,*b*, 2002, Nassis *et al.* 2001). Indeed, the human prostate expresses angiotensinogen, renin, ACE, AngII, AT_1 receptors and low amounts of AT_2 receptors. While mRNA of angiotensinogen and renin are detected in the human prostate (Fabiani, unpublished observations), AT_1 receptors were localized predominantly to periurethral stromal smooth muscle (Dinh *et al.* 2001*a*). The regulatory enzymes ACE and renin are localized to the epithelium. AngII immunoreactivity has been localized to the basal layer of the epithelium (Dinh *et al.* 2002). In addition, binding sites for AT_4 receptors have been found in the prostate, localized to the glandular epithelium, as evidenced by an autoradiographic study (Dinh *et al.* 2001*b*). It is not known if these binding sites are identical to insulin-regulated aminopeptidase, as recently reported for brain AT_4 receptors (Albiston *et al.* 2001).

Regulation and functions of local RAS

The functions discussed in this section are summarized in Fig. 1. There are considerable data for the regulation of RAS components in male reproductive tissues. Testicular renin, ACE, angiotensinogen and total AT receptors increase concomitantly with plasma gonadotropins at the onset, or immediately before, puberty (Hohlbrugger *et al.* 1982, Parmentier *et al.* 1983, Speth *et al.* 1999). Interestingly, AngII has been shown to inhibit Leydig cell function and is thus implicated in the local regulation of the testis by

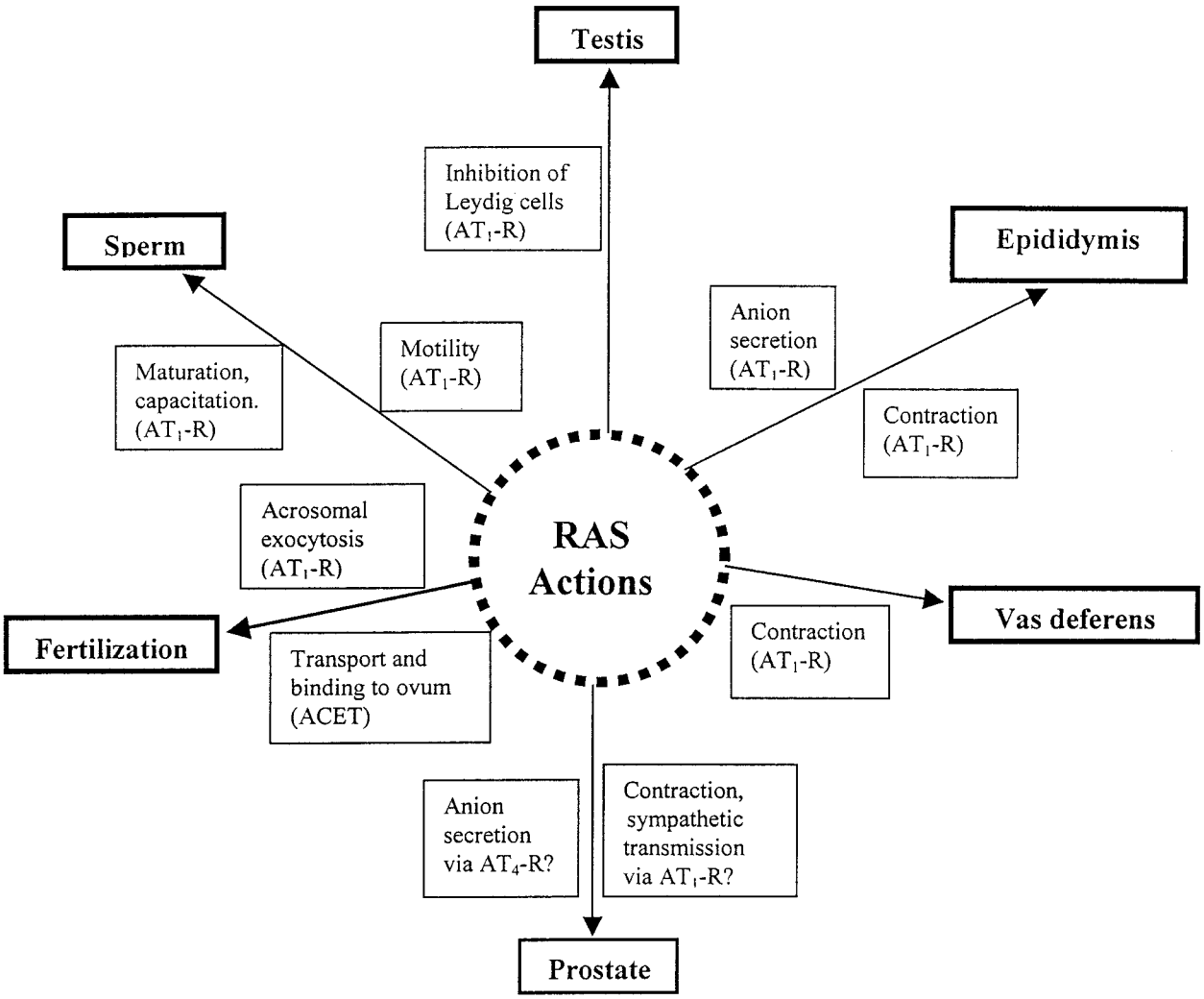

Figure 1 A summary of the functions of the local RAS in the male reproductive system. ?, indicates uncertainty. AT_1-R, angiotensin receptor subtype 1; AT_4-R, angiotensin receptor subtype 4.

pituitary luteinizing hormone (Khanum & Dufau 1988). In that study, AngII was found to inhibit adenylate cyclase activity in Leydig cell membranes and reduce basal and human chorionic gonadotropin-stimulated cAMP as well as testosterone production. These data suggest that locally produced AngII could negatively modulate luteinizing hormone stimulation of Leydig cells.

The association of RAS and hormone, such as testicular hormonal regulation of RAS, has been further demonstrated in other sites in male reproductive organs. In the epididymis, renin-like activity has been shown to be androgen-dependent (Uchendu 1995); more recent reports show that the expression of RAS, particularly at the levels of its precursor angiotensinogen and its AT_1 receptor, is predominantly testosterone-dependent (Leung *et al.* 2000). The expression of epididymal AT_1 and AT_2 receptors differs in mature and immature rats, with AT_2 receptor being expressed predominantly in immature rats (Leung *et al.* 1998*a*). These differences may imply that the AT_2 receptor mediates growth and differentiation in the immature male reproductive system and appears not to be involved in the secretory activity in the mature epididymis.

Grove & Speth (1989), in a dramatic display of expulsive power by the isolated epididymis, showed the contractile action of AngII. The AT_1 receptor has been shown to mediate directly the anion

secretion by the epididymis, a process which is predominantly dependent on testicular testosterone (Leung *et al.* 2002). The action of AngII on anion secretion is mediated by the stimulation of the prostaglandin PGE_2 (Leung *et al.* 1998*b*). The expression of cyclooxygenase, a principal enzyme in prostaglandin synthesis, is controlled by testicular androgens (Wong *et al.* 1999, Cheuk *et al.* 2000). These data suggest that androgens control anion secretion in the epididymis by stimulating components of the RAS and of prostaglandin synthesis. Disruptions of the RAS or prostaglandin pathways are therefore potential sites for the management of epididymal functions, thus affecting sperm maturation and expulsion.

In addition to hormonal regulation, local RAS is also responsive to various stresses and pathological conditions (Leung & Carlsson 2001). In the epididymis, chronically hypoxic stress attenuates the transcriptional and post-transcriptional expression of angiotensinogen, and by implication, the local production of angiotensins. Since AngII is involved in epithelial anion secretion (Leung *et al.* 1997*b*), this down-regulation would be expected to decrease anion and thus fluid secretion by the epididymis, which would in turn lead to the disturbances in testicular function observed in chronic hypoxia (Leung *et al.* 2001).

The prostate has been the latest site of investigation for a local RAS. In a series of publications from the same laboratory (Dinh *et al.* 2001*a*,*b*, 2002, Fabiani *et al.* 2001, Nassis *et al.* 2001), all components of the RAS in the human prostate, including angiotensinogen, renin, ACE, AT_1 receptors as well as AngII itself, were identified and localized to the glandular epithelium (ACE, AngII, AT_4 receptor) and periurethral stromal smooth muscle (AT_1 receptor). The localization of these components, and the stimulation of noradrenaline release from prostatic nerve endings by AngII, suggest roles in anion secretion, tubular contractility and the enhancement of sympathetic nerve activity (See Fig. 1). Furthermore, the RAS was found to be overactive in benign prostatic hyperplasia (BPH). Interestingly, renin and ACE protein at both the gene and protein levels, as well as AngII, are increased, while AT_1 receptors are down-regulated in BPH. These findings show an association of local RAS activity with a common clinical condition, suggesting a potential therapeutic approach by the inhibition of the local RAS. On the other hand, the presence of AT_4 receptors also opens up the way to further studies in this new pathway for angiotensin action.

RAS and male fertility

The importance of local RAS for male fertility is presently unclear, and to some extent, debatable. Gur *et al.* (1998) showed AT_1 receptors in the tail of non-capacitated sperm, and head-to-tail distribution in capacitated sperm. AngII induced acrosomal exocytosis only in capacitated sperm. AngII has been shown to maintain sperm motility via an AT_1 receptor-mediated mechanism (Vinson *et al.* 1995, 1996). However, some studies with ACE inhibitors or AngII receptor antagonists have reported no effect on fertility in rats (Dostal *et al.* 1991, Spence *et al.* 1995). Although the ACE inhibition does not exhibit a direct effect on the capacitation process or acrosome reaction, decreased binding of human spermatozoa to oocytes after inhibition by captopril may indicate that kininase II is involved in sperm–egg interactions (Kohn *et al.* 1998*b*). In addition, knockout mice lacking ACE show infertility of the males only (Krege *et al.* 1995). Restoring ACET corrected the infertility, while sperm expression of the sACE did not, demonstrating the selective dependence of male fertility on ACET (Ramaraj *et al.* 1998, Kessler *et al.* 2000). The defect appears to be poor sperm migration in the oviduct and a failure of ovum penetration. The failure of sACE replacement to correct the infertility and the normal fertility of angiotensinogen knockout mice (Kim *et al.* 1995) indicate that the absence of angiotensins is not the crucial block and that some other ACET-specific substrate is involved (Kessler *et al.* 2000). However, this view is challenged by a more recent study in angiotensinogen-deficient mice by Tempfer *et al.* (2000), which showed a decreased fertility attributable in part to male infertility. Interestingly, decreased *ex utero* survival rate is observed in mutant mice when both AT_1 receptor subtypes (AT_{1a} and AT_{1b}) are knocked out, which is quantitatively similar to that of the angiotensinogen knockout (Tsuchida *et al.* 1998, Doan *et al.* 2001). These data suggest that major biological functions of endogenous AngII elucidated by the abnormal phenotype of angiotensinogen knockout mice are mediated by the AT_1 receptors.

The above results would seem to relegate the local RAS to a subsidiary role in fertility. However, these results could be explained by the presence of a blood–testis barrier, which can be a significant block to the entry of drugs (see discussion in Vinson *et al.* 1997). Hence, at present the experimental evidence for a definitive role for the RAS in fertility is controversial. On the hand other, the effect of RAS blockade on a local RAS activity in other sites in the male reproductive tract has been investigated. In the epididymis, for example, electrophysiological studies using a short-circuit current technique demonstrated a stimulatory effect of AngII on the epididymal electrogenic ion transport. This effect was inhibitable by the addition of AT_1 receptor antagonist, losartan, but not by AT_2 receptor antagonist, PD123177, indicating a functional role of AT_1 receptor in epididymal function (Leung *et al.* 1997*b*, 2002). In the prostate, blockade of AT_1 and AT_2 receptors inhibited AngII-stimulated sympathetic transmission. These data provide direct evidence in support of a functional role for the local RAS in modulating sympathetic transmission in the prostate, which may have important implications for the pathophysiology of BPH (Fabiani *et al.* 2001). Interestingly, the effects of AngII infusion and ACE inhibition on the RAS activity have been also examined in the testes. In that study, infusion of AngII decreased the local synthesis of testicular ACE mRNA (Schunkert *et al.* 1993), indicating that a local RAS activity is responsive to RAS inhibition. Nevertheless, the intrinsic RAS activity in male reproductive organs, and thus its implications for male reproduction, await further investigation.

Conclusions

The data reviewed above and summarized in Table 1 and Fig. 1 provide substantial evidence for local AngII production in various parts of the male reproductive system. While details of the pathways of AngII production need attention, the major issues of identification and localization are largely known. The detection of AT_4 receptors in the prostate has brought into focus the need to consider the involvement of other angiotensin peptides besides AngII. The functions and regulation of local RAS are being elucidated: in particular (i) the importance of sperm-specific ACE, AT_1 receptor and angiotensinogen in sperm function and fertility, (ii) the involvement of a local RAS in the epididymis, a core segment for the maturation of sperm, and (iii) the likely involvement of the RAS in BPH. Future investigations in these areas are the most likely to lead to a fuller understanding of, and practical applications in, male fertility and disease.

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