REVIEW ARTICLE

Purinergic signalling in the reproductive system in health and disease

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Abstract There are multiple roles for purinergic signalling in both male and female reproductive organs. ATP, released as a cotransmitter with noradrenaline from sympathetic nerves, contracts smooth muscle via P2X1 receptors in vas deferens, seminal vesicles, prostate and uterus, as well as in blood vessels. Male infertility occurs in P2X1 receptor knockout mice. Both short- and long-term trophic purinergic signalling occurs in reproductive organs. Purinergic signalling is involved in hormone secretion, penile erection, sperm motility and capacitation, and mucous production. Changes in purinoceptor expression occur in pathophysiological conditions, including pre-eclampsia, cancer and pain.

Synopsis

Male reproductive organs

Penis and penile erection
Testis
Vas deferens
Seminal vesicles
Epididymis
Prostate
Sperm

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Female reproductive organs

Ovary
Oocytes
Fallopian (uterine) tubes/oviduct
Uterus
  Myometrium
  Endometrium
Cervix
Amnion
Placenta
Vagina
Mammary glands

Conclusions

There is increasing recognition for purinergic signalling in both male and female reproductive organs, which have not been reviewed recently. For full accounts of cancer of various reproductive organs see [62].

Male reproductive organs

Smooth muscle function and secretion in the organs of the male reproductive system are under autonomic nervous control. The seminal vesicles, prostate and related glands as well as the vas deferens and penile vessels are innervated by short sympathetic neurons in the pelvic plexus. There are earlier reviews that include discussion of the involvement of nucleotides and nucleosides in the complex innervation of the male reproductive system ([14,157]; see also [66]). There are also studies of P2X receptor immunoreactivity in male reproductive organs [227] and of the localisation of plasma membrane-bound NTPDases [260]. A high expression and activity of ecto-5′ nucleotidase (CD73) in the male murine reproductive tract has also been reported that may impact male fertility [261].

Penis and penile erection

The actions of adenosine 5′-triphosphate (ATP) on the corpus cavernosum of a wide spectrum of different mammalian species were first described in 1997 [209]. ATP can induce significant relaxations of rabbit cavernosal tissue which was unaffected by removal of the endothelium [402]. ATP has a powerful relaxant effect on corporal smooth muscle at high tone or pre-stimulated tissue, but causes contractions at low tone [443]. Both human and rabbit corpus cavernosal pre-contracted strips relaxed to ATP [232]. The powerful relaxant effect of ATP on the smooth muscle of the dog, rabbit and human corpus cavernosum is about the same as that produced by nitric oxide (NO), and it was suggested that a combination of ATP with an NO donor might be an effective therapeutic approach for erectile disorders [172]. The relaxant effect of ATP and adenosine was greater in mature (24 months old) rabbits than at 3 and 7 months [332].

Intracavernosal injection of adenosine induced a full erection in cats [394,395]. Adenosine also had prejunctional inhibitory actions on excitatory sympathetic cotransmission to the rabbit corpus cavernosum, probably acting via A_{2B} receptors [79]. The effect of long-term testosterone propionate therapy on endothelium-dependent and -independent relaxations of corpus cavernosum strips to purines was examined [192]. It was shown that the NO synthase (NOS) inhibitor, nitro-L-arginine methylester (L-NAME), inhibited the relaxations to adenosine 5′-diphosphate (ADP), but not to ATP or adenosine (suggesting that $P2Y_1$ receptors on endothelial cells might be involved). Relaxations in response to ADP (but not ATP or adenosine) were significantly enhanced by testosterone therapy. In fact, $P2Y_1$ receptor transcripts were later identified on the endothelial cells which line the lacunar space and blood vessels in the penis, but not on corpus cavernosum smooth muscle cells [308]. Functional studies showed that the $P2Y_1$ selective agonist, adenosine-5′-(β-thio)-diphosphate, relaxed the human corpus cavernosum via endothelial release of NO [364]. Nerve-mediated relaxation of human corpus cavernosal smooth muscle strips pre-contracted with phenylephrine is amplified by stimulating $P2Y_1$ and $P2Y_2$ receptors, suggesting a purinergic relaxing mechanism separate from the endothelium NO-mediated relaxing pathway [158]. ATP (in contrast to ADP) acts as a potent and NO-independent relaxant agent of human and rabbit corpus cavernosum, although part of its relaxant effect is attributable to adenosine, after breakdown of ATP, acting via A_{2A} receptors [122].

Two distinct layers of smooth muscle were identified in the penile bulb of rats, an inner layer (parenchyma) and an outer sheet (sac). Transmural stimulation initiated non-adrenergic, non-cholinergic (NANC) inhibitory junction potentials in parenchymal muscle, but excitatory junction potentials (EJPs) in the sac smooth muscle [161]. It was claimed that ATP released from nerves produces relaxation of cavernosum smooth muscle via $P2Y_4$ receptors on smooth muscle, while ADP acting via $P2Y_1$ receptors on endothelial cells produces relaxation via NO [72]. Modulation of cavernosum smooth muscle relaxation occurs by activation of $P2Y_6$ receptors via non-neuronal and non-NO-dependent mechanisms [224]. It was suggested that ADP-sensitive $P2Y_{12}$ and/or $P2Y_{13}$ receptors might mediate relaxation of corpus cavernosum through the release of prostanoids [112].

Both ATP and adenosine produced penile tumescence in anaesthetised dogs, probably via adenosine A_{2A} receptors; pelvic nerve stimulation also produced tumescence, but this did not appear to be via A_{2A} receptors [307]. Experiments on isolated rabbit corpus cavernosum suggested that A_{2B} receptors were also involved in relaxation [189]. In another paper, it was claimed that adenosine directly relaxes cavernosal smooth muscle via both A_{2A} and A_{2B} receptor subtypes, while it modulates sympathetic neurotransmission via A_1 receptors [404]. Activation of A_1 receptors during penile erection results in reduced noradrenaline (NA) release and reduced cavernosal smooth muscle contraction, thereby facilitating penile erection [303]. Impaired adenosine signalling via A_1 receptors has been claimed to contribute to erectile dysfunction [304]. The possibility has been raised that elevated adenosine signalling contributes to priapism, a condition of persistent penile erection lasting at least 4 h in the absence of sexual excitation [86,305]. Excess adenosine in penile erectile tissue in mice lacking adenosine deaminase contributes to priapism via A_{2B} receptor signalling [272]. A recent review highlights adenosine signalling pathways operating in penile tissue as significant therapeutic targets for the treatment of erectile disorders [430].

ATP was shown to dilate isolated bovine and canine penile arteries [50]. However, EJPs were recorded in smooth muscle cells of both penile artery and vein in response to nerve stimulation [206], resembling the ATP-mediated EJPs recorded in the vas deferens.

The incidence of erectile dysfunction among patients with renal failure is significantly higher than in the general population. Clinical complaints include an inability to initiate and maintain an erection. The purinergic relaxation responses in an experimental rabbit model of chronic renal failure were examined and the adenosine- and ATP-induced relaxations were not impaired [203]. Corpus cavernosum from men with vasculogenic impotence is partially resistant to adenosine relaxation due to endothelial A_{2B} receptor dysfunction [110]. Activation of A_{2B} receptors on endothelial cells contributes to penile erection via PI_3K/AKT signalling cascade-mediated endothelial NOS activation [432]. It was shown in a study of alloxan-induced diabetic rabbits that there was impairment of both direct $P2Y_4$ receptor-induced muscle relaxation and endothelium-dependent ADP $P2Y_1$ receptor-mediated relaxations [72]. The possibility that the responses of the vas deferens to ATP via P2X1 receptors are heightened in

overactive ejaculatory reflex associated with premature ejaculation has been considered [1].

Hypothyroidism, where testosterone levels are low, has been shown to lead to impotence in some men. In an experimental rabbit model of hypothyroidism, it was shown that relaxation responses to ATP, α,β-methylene ATP (α,β-meATP) and electrical field stimulation of corpus cavernosum strips pre-treated with phenylephrine were reduced significantly, while relaxations to adenosine were unchanged [448]. Impaired erectile function has been reported in CD73-deficient mice that resulted in reduced endogenous penile adenosine production [431]. Human corpus cavernosum from men with vasculogenic erectile dysfunction exhibit decreased ectonucleotidase NTPDase/CD39 activity leading to persistent extracellular ATP accumulation [111]. As a consequence, alteration of vascular responses of strips of corpus cavernosum from impotent patients to stable ATP analogues may be due to P2 purinoceptor desensitisation. In an earlier paper, this group showed that endothelial dysfunction in men with vasculogenic erectile dysfunction is associated with the loss of adenosine A_{2B} receptor activity in penile vessels [110]. In a later study by Faria and colleagues, it was shown that relaxation of human corpus cavernosum by P2 receptor agonists was severely attenuated in erectile dysfunction patients [113]. They suggest further that relaxation in control subjects may be mediated by ADP-sensitive $P2Y_{12}$ receptors, with a shift towards activation of $P2Y_1$ and perhaps $P2Y_{13}$ receptors in erectile dysfunction patients. ATP release from cavernosal tissue is greater in patients following prostatectomy as compared to patients with organic erectile dysfunction [202]. Severe bladder dysfunction secondary to chronic partial bladder outlet obstruction induced morphological, physiological and molecular dysfunction of corpus cavernosum smooth muscle, including significantly decreased relaxation responses to nerve stimulation and to ATP [240].

ATP can contract corpus cavernosum smooth muscle by activating P2X receptors, while it evokes relaxation via $P2Y_1$ and $P2Y_2$ receptors in the diabetic rat [158]. It was suggested that activating ATP based pathways can restore erectile function when NO bioavailability is impaired by diabetes.

Testis

Sperm cells are generated in the testis. Adenosine A_1 receptors were identified in rat testis using labelling with ^{3}H-cyclohexyladenosine (CHA) [296]. Steroid production in isolated Leydig cells was induced by adenosine [347]. The rat testes contain a dense population of A_1 receptors mediating inhibition of adenylate cyclase activity [387]. Binding studies with CHA in rat testes showed that A_1 receptors were localised in Sertoli cells of the seminiferous tubules [284]. A later study by this group showed that treatment of cultured Sertoli cells with pertussis toxin reversed the adenosine-mediated inhibition of cyclic AMP (cAMP) accumulation and potentiated the cAMP response to follicle stimulating hormone (FSH) [185,285]. A low affinity binding site for N^6-substituted adenosine derivatives in a rat testis membrane preparation showed the typical pharmacological profile of the cloned rat A_3 receptor [265]. The TM4 cell line is derived from a primary culture of Sertoli cells isolated from mouse testis. Selective agonists to both A_1 and A_2 receptors inhibited proliferation of the TM4 cells [362].

The distribution of P2X receptor subtypes in the rat testis was examined immunohistochemically [145]. P2X1, P2X2, P2X3 and P2X5 and P2X7, but not P2X4 and P2X6, receptors were localised in the testes. Blood vessels displayed P2X1 and P2X2 receptor immunoreactivity. P2X2, P2X3 and P2X5 receptors were expressed in the various germ cell types throughout the different stages of the cycle of the seminiferous epithelium. P2X4 receptor mRNA was expressed in the rat testis [44]. Sertoli cells also showed differential staining for P2X2 and P2X3 receptors during the cycle of the seminiferous epithelium, while P2X7 receptor expression was present throughout all stages. It was suggested that purinergic signalling may play a role in controlling the maturation of germ cell subsets of different developmental ages that exist alongside each other in the adult testis.

The Sertoli cells from the mammalian testis release several proteins and fluid into the lumen of the seminiferous tubules to play a key role in germ cell development. The main messenger of the response of immature Sertoli cells is FSH. A fast and biphasic increase in $[Ca^{2+}]_i$ was evoked when Sertoli cells were exposed to ATP [221]. P2 receptors on Sertoli cells are associated with phosphoinositide turnover and are activated by ATP and uridine 5′-triphosphate (UTP) suggesting that $P2Y_2$ or $P2Y_4$ receptors are involved. ATP and UTP had profound effects on FSH responsiveness [123]. Rapid and large accumulation of inositol 1,4,5-trisphosphate ($InsP_3$) in primary cultures of rat and mouse Sertoli cells were evoked by ATP, in keeping with $P2Y_2$ or $P2Y_4$ receptor activation [355]. ATP depolarises and produces an increase in $[Ca^{2+}]_i$ from intracellular stores in rat Sertoli cells, consistent with P2Y receptor activation, but it also induces a selective Na^+ influx from the extracellular medium consistent with activation of P2X receptors [126]. Prolonged treatment of cultured Sertoli cells with purinergic agonists for A_1 and P2 receptors led to an increase in aromatase, γ-glutamyl transpeptidase activities and transferrin secretion [271]. Oestradiol secretion in rat Sertoli cells was produced by extracellular ATP via both P2X and P2Y receptors, which led to increases in both $[Ca^{2+}]_i$ and $[Na^+]_i$ and membrane depolarisation [351]. mRNA for $P2Y_1$, $P2Y_2$ and P2X4 and P2X7 receptors was expressed in cultured rat Sertoli cells [214]. It has been claimed that mitochondria are essential components of Sertoli cell signalling that control purinergic Ca^{2+} responses, mediated by P2X2 and $P2Y_2$ receptors [414].

During spermatogenesis, germinative cells are supported by Sertoli cells which have their secretory activity precisely regulated by germinative and myoid peritubular cells of the seminiferous tubules that make up the bulk of the testis. Extracellular ATP and its breakdown product adenosine are both involved in this process after ATP and adenosine are released from Sertoli cells during paracrine regulation of the maturation process [141]. Extracellular inosine increases ERK1/2 and p38 phosphorylation in Sertoli cells, possibly through A_1 receptor activation [383]. Sertoli cells are key cells in the development and maintenance of stem cell spermatogenesis as well as in the secretion of ATP activated Cl^-- and K^+-rich fluid into the lumen of seminiferous tubules [20]. Extracellular inosine participates in tumour necrosis factor-α (TNF-α)-induced NO production in cultured Sertoli cells [89]. EctoATPases and adenosine deaminase have been identified and characterised in rat Sertoli cells [26,73].

Leydig cells, lying between the seminiferous tubules in the testis, secrete androgens in response to luteinising hormone (LH) from the anterior pituitary gland. Activation by ATP of rat Leydig cells stimulates testosterone secretion via a mechanism dependent on the influx of Ca^{2+} from the external medium [128], implying mediation via a P2X receptor subtype. The pharmacological features of the P2X receptor involved were most similar to the P2X2 subtype [324]. Leydig cells produce androgen, which is dependent on androstenedione, the precursor of testosterone synthesis and on activation of the microsomal enzyme 17β-hydroxysteroid dehydrogenase (17βHSD). ATP generation is via the glucose transport system, which is required for the activation of 17βHSD in the last step of androgen biosynthesis [200]. Extracellular pyridine dinucleotides and adenosine modulate the activity of 17βHSD [120]. Sympathetic innervation of human Leydig cells and its influence on the secretion of testosterone probably involves ATP release as a cotransmitter with NA [126].

In patients with cystic fibrosis transmembrane regulator (CFTR)-mediated infertility with azoospermia or severe oligozoospermia, change in the gene expression of the ATP-binding cassette superfamily transporter is involved [223].

Important regulators of the male reproductive system are thyroid hormones, which modulate the extracellular ATP levels in hypothyroid cultured Sertoli cells. The effect of congenital hypothyroidism and thyroid hormone supplementation on NTPDase activities in Sertoli cells influences the actions of adenosine and ATP on reproductive functions throughout development [455].

Germ cell death is part of the regulation of normal testicular function and disruption of this orderly process is associated with several male reproductive disorders. It was considered that the mitochondrial ATP production machinery plays an important role in regulating primary pathways of human male germ apoptosis, although there seems to be a secondary pathway of testicular apoptosis that does not require mitochondrial

ATP production [106] and perhaps involves P2X7 receptors. The antiviral drug, acyclovir, a synthetic purine nucleoside analogue, induces testicular toxicity by adverse effects on the sperm parameters and serum level of testosterone in male rats [293].

Sympathetic and sensory nerves supply the rodent testicular artery and the pampiniform plexus, a venous network that surrounds it. Innervation is largely confined to the capsule of the testes and superficial blood vessels, suggesting a role in the control of temperature. Smooth muscle is present in the testicular capsule of the rat, mouse, rabbit and man. ATP, released as a cotransmitter from sympathetic nerves, stimulates contraction of testicular smooth muscle, probably via P2X1 and/or P2X2 receptors [23]. Using Western blots it has been shown that mouse Leydig cells express P2X2, P2X4, P2X6 and P2X7 receptor subunits and heteromeric P2X2/4/6 receptors may also be present [17].

Vas deferens

The vas deferens is an important organ of the male reproductive system mainly concerned with transporting sperm from the testes to the penis during ejaculation. The vas deferens store mature spermatozoa and is responsible for dispersing sperm into the urethra to be combined with other components of sperm prior to ejaculation. The sympathetic nerve-vas deferens preparation, introduced by Huković in [171] has been used as a model to study autonomic neuromuscular transmission ([64,65]; see [60,67,331,435]).

Burnstock and Holman [63–65] studied the electrophysiology of sympathetic neurotransmission in the guinea-pig vas deferens. They showed that EJPs in smooth muscle in response to single nerve pulses summed and facilitated, until at a critical depolarisation threshold spikes were initiated associated with contraction. However, they were puzzled that adrenoceptor antagonists did not abolish the EJPs, NA being established as the sympathetic neurotransmitter at that time. Over 20 years later, it was recognised that ATP, acting as a cotransmitter with NA, was responsible for the EJPs, which were blocked by the ATP receptor desensitiser α,β-meATP [375]. David Westfall and colleagues were the first to demonstrate that ATP produced contraction of the vas deferens when released as a cotransmitter with NA from sympathetic nerves [114,377,434]. The non-adrenergic contractile component of the responses to sympathetic stimulation was antagonised by arylazido aminopropionyl ATP [115], suramin [102,258,374,422], pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS; [266]), NF023 [378] and desensitised by α,β-meATP both in vitro and in vivo [12,57,268,375]. 6-Hydroxydopamine (6-OHDA) abolished both adrenergic and purinergic components, supporting the view that they were cotransmitters in sympathetic nerves (Fig. 1; [9]). ATP and its breakdown products ADP, adenosine
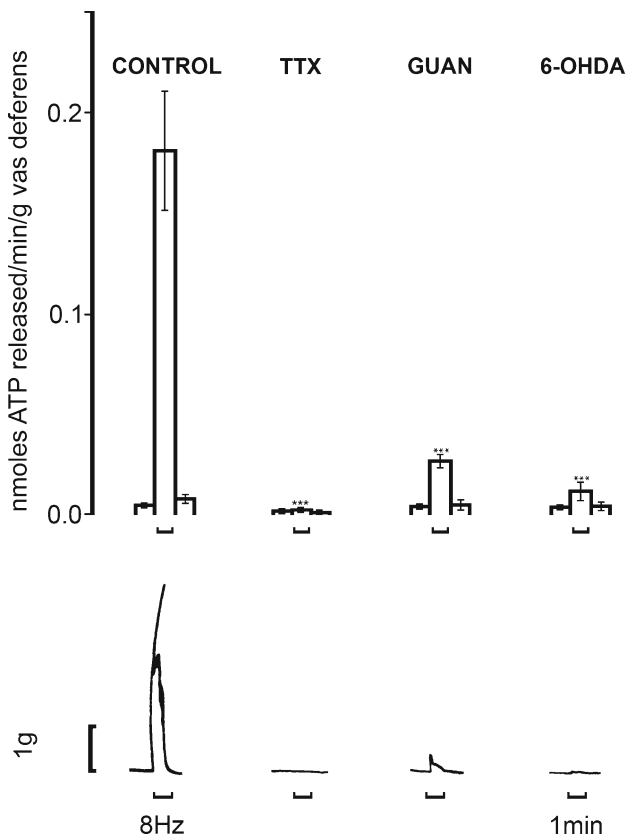
Fig. 1 Release of endogenous ATP from control ($n=32$), 6-OHDA-pretreated ($n=7$), TTX- (16 μM, $n=7$) and guanethidine (GUAN)-exposed (5 μg/ml, $n=7$) guinea-pig vasa deferentia during field stimulation at 8 Hz (0.5 ms, 20 V). *Upper panel*: mean ± SEM nmol of ATP released/min per g of vas deferens. *Lower panel*: contractile responses to field stimulation. The vasa deferentia were stimulated for 1 min as denoted by the upward bracket. ***$P<0.001$. (Reproduced from [208], with permission from Elsevier)

Fig. 2 Release of endogenous ATP from control ($n=32$) and reserpine-pretreated ($n=12$) guinea-pig vasa deferentia during field stimulation at 8 Hz (pulse width 0.5 ms, 20 V). *Upper panel*: mean ± SEM nmol of ATP released/min per g of vas deferens. *Lower panel*: contractile responses to field stimulation. The vasa deferentia were stimulated for 1 min as denoted by the upward bracket. Note that the second slow phase of the mechanical response has gone in the reserpine-pretreated tissue. (Reproduced from [208], with permission from Elsevier)

monophosphate (AMP) and adenosine were detected in the surfusate during transmural stimulation of nerves in the vas deferens [234,235]. As an alternative explanation Neild and Hirst proposed in the mid 1980s that EJPs were due to NA acting on hypothetical γ-adrenoceptors [301]. This was much debated at the time [35,166,376]. However, it was shown that NA, unlike ATP, did not mimic the EJP [377] and that reserpine, which depleted NA but not ATP, failed to affect the rapid component of sympathetic nerve-modulated responses (Fig. 2; [208]), and the γ-hypothesis was abandoned. Direct evidence was presented for concomitant release of NA, ATP and neuropeptide Y (NPY) from sympathetic nerves supplying the guinea-pig vas deferens [187]. A more recent paper described a purinergic component of sympathetic nervous control of the human vas deferens [22]. Rapid emission of sperm into the urethra prior to ejaculation is mediated by the fast purinergic component of the contraction, while the sustained noradrenergic contraction prevents any reflux into the vas deferens during ejaculation. Electrophysiological studies of the vas deferens to study packaged release of ATP from
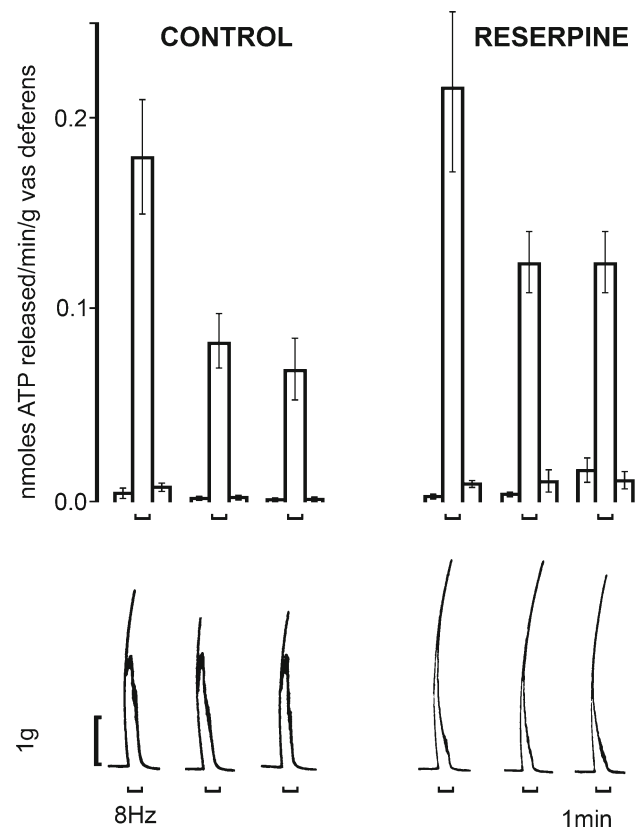
sympathetic nerve varicosities have been carried out (see [34,41,51,53,388,452]). Secretion of transmitters from a single varicosity was shown to be intermittent, with only a small percentage of varicosities releasing transmitters during sympathetic nerve stimulation. All varicosities secrete ATP [186], release is quantal [226] and Ca^{2+}-dependent [251]. There is regional variation, with dominance of purinergic signalling at the prostatic segment of the vas deferens, while noradrenergic signalling was more prominent in the epididymal segment (Fig. 3a and b) [97,211,345].

Neuromodulation of transmitter release is via prejunctional A_1 adenosine receptors [255,398] as well as receptors to NA (see [400]), nicotine, γ-aminobutyric acid, NPY, histamine, prostaglandins, angiotensin, opioids, calcitonin gene-related peptide and cannabinoids (see [67,99]). There is postjunctional synergism by the sympathetic cotransmitters NA and ATP [165,201]. NA potentiates the contractile responses of the vas deferens to ATP via a protein kinase C (PKC) mechanism that involves the inhibition of myosin light chain phosphatase and subsequent calcium sensitisation
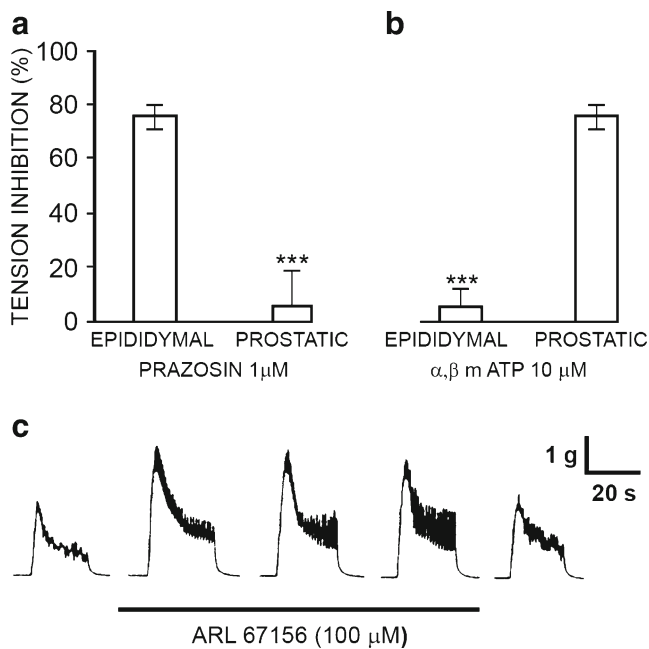
Fig. 3 **a** and **b** Antagonism of adrenergic and purinergic activity. **b** Inhibition of the electrically evoked twitches after incubation of the tissues for 10 min with 10 μM α,β-mATP. **c** Inhibition of the electrically evoked twitches after incubation of the prostatic (*n* =10) and epididymal (*n* =8) segments with 1 μM prazosin for 20 min. Bars represent SEM. *P <0.005. Seven separate prostatic and epididymal segments of rat ductus were utilized. Symbols as in **b**. (Modified from [345], with permission from Elsevier.) **c** The time course of the effect of ARL 67156 on neurogenic contractions evoked by trains of pulses for 20 s at 2 Hz. The trace shows contractions recorded at 10-min intervals. ARL 67156 (100 μM) was added immediately after the end of the control response (*first panel*), left in contact with the tissue for 30 min (*second*, *third* and *fourth panels*), then washed out and stimulation repeated (*fifth panel*). (Reproduced from [436], with permission from John Wiley & Sons)

[373]. Prejunctional P2Y receptors have also been shown to inhibit, while P2X receptors facilitate transmitter release [330]. ATP and NA appear to be released from different populations of vesicles (see [104]). Postjunctional neuromodulation also occurs [96,290].

Ectonucleotidase activity has been described on smooth muscle membranes of the vas deferens, including 5′-nucleotidase [219,274]. Sympathetic purinergic neurotransmission was enhanced by the ecto-ATPase inhibitor, ARL67156, in the guinea-pig vas deferens (Fig. 3c; [143,436]). Soluble nucleotidases were shown to be released together with transmitters from sympathetic nerves supplying the vas deferens as a novel mechanism for neurotransmitter inactivation [399,437].

The main smooth muscle receptor to ATP is the P2X1 ion channel receptor, leading to increase in intracellular calcium and the fast component of contraction. P2Y$_2$ receptors mediating contraction of the rat vas deferens have also been claimed [58], while P2Y$_1$ receptors mediate relaxation to ATP [47]. Nifedipine blocked P2X-mediated responses to ATP, but not to NA [250,389]. Clusters of P2X1 receptors

on smooth muscle opposite close sympathetic terminal varicosities were described in the mouse vas deferens [27]. P2X1 receptor internalisation has been reported after exposure to the agonist α,β-meATP [105], perhaps underlying the mechanism of desensitisation. Perinuclear immunoreactivity for P2X7-like receptors has been reported in smooth cells of the guinea-pig vas deferens [270]. It has been claimed that P2X2 receptors expressed by interstitial cells of Cajal in vas deferens are involved in semen emission [68].

During developmental, changes in purinergic signalling in the vas deferens occur later than in the gut, because rats are not sexually active until about 10 weeks, although the muscle morphology of the vas deferens appeared to be mature by day 35 [131]. EJPs in response to nerve stimulation and ATP were not observed in the vas deferens of mice of less than 18 days postnatal [139]. Another early study showed that at 3 weeks postnatal in the rat (the earliest time studied) the responses of the vas deferens to field stimulation with single or trains of pulses lacked the adrenergic component, although the non-adrenergic (purinergic) component was present [248]. Responses to ATP first appeared at day 15 and increased with age [169]. Adenosine, acting via prejunctional A$_1$ receptors, inhibited nerve-mediated contractions when they were first seen at day 15, but its actions decreased with age [169]. Inhibitory postjunctional A$_2$-like receptors and prejunctional A$_1$ receptors were present from days 10 and 15, respectively ([318]; Fig. 4). They also identified postjunctional *excitatory* A$_1$ receptors that only appeared after day 20. Expression of P2X receptors also occurred at day 20 [168]. Changes in sympathetic nerve-evoked contractions of the *circular* muscle layer of the guinea-pig vas deferens showed a significant decrease with increasing age, apparently due to postjunctional rather than prejunctional mechanisms; responses to α,β-meATP decreased in parallel [338]. An increase in P2X1 receptor mRNA expression was shown between postnatal days 10 and 42 [238]. Both pre- and post-junctional mechanisms caused the maturation of fast purinergic junctional transmission of the longitudinal muscle of the mouse vas deferens between 21 and 42 days postnatal [239].
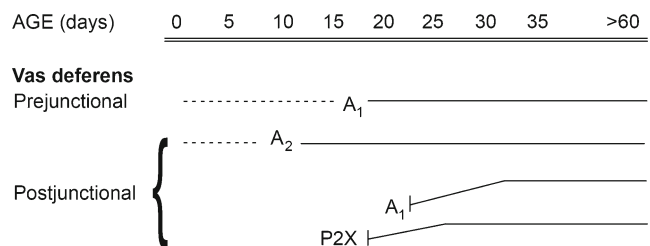

Fig. 4 Diagram summarizing the development of functional responses mediated by purine receptors in the rat vas deferens. The *dashed lines* represent ages at which it was not possible to study functional responses, and the *solid lines* show when responses were observed, with the slope of the line indicating whether a response, in general, increased or decreased with age. (Modified and reproduced from [168], with permission from Elsevier)

By analogy with the release of transmitters from endothelial cells lining blood vessels (see [61]) and urothelial cells in bladder and ureter [212,421], it was shown that prostaglandin E_2 was released from epithelial cells of the rat vas deferens in response to neurally released ATP acting via P2Y receptors to mediate neurogenic contractions [353].

Data about the role of ATP as a cotransmitter with NA in the vas deferens from humans is conflicting. Early studies suggested that sympathetic neuromuscular transmission was largely by NA [16,38,163]. However, staining with quinacrine, which indicates high ATP levels, was described in nerve terminals in human vas deferens [11] and the involvement of both P2X1 receptors and α_1-adrenoceptors in neurotransmission was reported [22]. A recent paper has shown that there is purinergic transmission to the longitudinal, but not circular muscle, which might explain some of the earlier ambiguities [13]. P2X1 receptor antagonists acting as non-hormonal male contraceptives has been proposed (see [101,294]), but the effectiveness of such drugs in man is not yet established. However, a purinergic cotransmitter pathway in the vas deferens of man appears to be present [101]. In P2X1 receptor-deficient mice, contractions of the vas deferens to sympathetic nerve stimulation were reduced by up to 60 % and there was a 90 % decrease in male fertility [294].

An enhanced initial fast component of cotransmission of the vas deferens in response to sympathetic nerve stimulation from spontaneously hypertensive rats (SHR) was described in 1985 [415]. This appears to be in keeping with reports of a significant increase in the purinergic component of cotransmission from sympathetic nerves supplying blood vessels of SHR (see [333]). A reduction in purinergic prejunctional neuromodulation via A_1 receptors in SHR has also been described [176] and other possible sites of adenosine malfunction in hypertension discussed [21]. In streptozotocin diabetic rats after 8 and 12 weeks, there was an increase in the purinergic component of the responses to sympathetic nerve stimulation in the vas deferens [427], although an earlier paper concluded that sympathetic neuropathy occurred in the vas deferens of the streptozotocin-diabetic mouse [188]. Chronic alcohol treatment differentially affects noradrenergic and purinergic responses in the rat vas deferens, perhaps modifying male reproductive tract function [49,197].

Seminal vesicles

Seminal vesicles are a pair of male accessory sex glands that open into the vas deferens before it joins the urethra. They secrete most of the liquid component of semen.

ATP was considered in an early paper as an excitatory transmitter in response to hypogastric nerve stimulation to the guinea-pig seminal vesicle [297]. EJPs in response to nerve stimulation were recorded in the circular muscle of the guinea-pig seminal vesicle, resembling those mediated by

ATP in the vas deferens [309]. Evidence was presented that ATP was involved as a cotransmitter in the hypogastric nerves supplying the guinea-pig seminal vesicles [269,320,424]. Endothelin (ET)-1 causes modest tonic contractions of the rat seminal vesicle via ET_A receptors and selectively potentiates the motor responses to ATP [246].

Epididymis

The epididymis is a highly convoluted tube about seven metres long in man that connects the testes to the vas deferens. Spermatozoa, entering the epididymis from the efferent ducts of the testes, undergo maturation as they progress through the epididymis in response to contractions.

ATP stimulates via P2 receptors short circuit currents in primary monolayer cultures of rat epididymal cells when applied to the apical, but not to the basolateral side of the monolayer and ATP included in the luminal perfusion solution caused an increase in water and Cl^- secretion [231,441]. The authors noted that spermatozoa contain high levels of ATP and it has been proposed that ATP released from spermatozoa may affect anion and fluid secretion by the epididymis. ATP-activated cation conductance in human epididymal cells may be responsible for secreting K^+ across the epididymal epithelia, resulting in a much higher K^+ concentration in the lumen of the epididymis than in the blood [74]. ATP activates both Ca^{2+} and cAMP-dependent Cl^- conductances in rat epididymal cells [75]. ATP is released from principal cells in the cauda epididymis of mice and evidence presented that the CFTR is involved in the release mechanism [354]. Since mutations of CFTR are a leading cause of infertility, the authors propose that defective ATP signalling in the epididymis might contribute to the dysfunction. It has been claimed that pannexins may play a role in ATP secretion into the epididymal lumen and basal extracellular spaces for functions involving sperm transport and maturation [405].

Evidence was presented that both ATP and NA act as cotransmitters in sympathetic nerves supplying the smooth muscle of the rat cauda epididymis involving P2X and α_1-adrenoceptors, respectively [416]. Prejunctional inhibitory neuromodulation of nerve-mediated contractions by P1 and possibly P2Y receptors was later proposed [417]. Both A_1 and A_{2A} receptor subtypes appear to be involved [162]. The sympathetic nerves supplying the cauda epididymis mediate the ejaculatory contraction at orgasm, with both cotransmitters NA and ATP involved. When α,β-meATP, which is a selective desensitiser of purinergic transmission, was injected directly into the cauda epididymis, fertility of male rats was impaired [335].

Using immunohistochemistry and RT-PCR, P2X1, P2X2, P2X4, P2X5 and $P2Y_1$ and $P2Y_2$ receptor subtype protein and mRNA were identified in the mouse epididymis and shown to mediate Cl^- fluid secretion from epididymal epithelium and

sperm maturation [365]. In a more recent paper, P2X3 and P2X6 receptor genes were also identified in rat epididymal epithelial clear cells as well as A$_1$, A$_{2B}$ and A$_3$ adenosine receptor genes [33]. They suggest that activation of these receptors may play a significant role in luminal acidification in the epididymis, a process that is crucial for the establishment of male fertility.

Prostate

The fluid secreted by the prostate during ejaculation consists of about a third of the volume of semen. Nerve stimulation responses of the smooth muscle of the prostate gland were inhibited by the prejunctional action of adenosine [327]. ATP and UTP caused an increase in outward current and hyperpolarisation of isolated rat prostate secretary epithelial cells, perhaps controlling exocrine secretions [204].

ATP is an excitatory cotransmitter with NA to the smooth muscle and fibromuscular stroma of the prostate gland of rat and guinea-pig [56,418]. The P2 receptor antagonist, suramin, attenuates nerve-mediated contractions of the guinea-pig prostate [225]. P2 receptors have been shown to be present in the human prostate, including P2Y$_1$ receptors [181] and P2X1 receptors [205,244]. P2X1 receptors were also expressed in the rat prostate [227]. Human prostate epithelial cells express P2X1, P2X2 and P2X7 receptors [371]. It was shown further that expression of these receptors is low in healthy men, but with the development of prostatic cancer their expression increases. Prostate expression of P2X1, P2X2 P2X5 and P2X7 receptors in rats decreases with age, while expression of P2X3, P2X4 and P2X6 receptors increases [369]. Ectonucleotidases have been identified in the human prostate [215]. Histamine, acting via H$_1$ receptors, potentiates nerve-mediated contractions of the guinea-pig prostate by postjunctional enhancement of the response to ATP [196]. ATP modulates the release of NA from sympathetic nerves supplying the prostate via two different prejunctional receptors, namely A$_1$ and P2Y receptor subtypes [289]. Activation of adrenergic receptors in the rat prostate triggers the release of ATP both in vitro and in vivo [295].

A$_{2B}$ receptors were claimed to be present on human prostatic stromal cells [83]. Adenosine A$_1$ and A$_{2A}$ receptors modulate α_1-adrenoceptor-mediated contractions of human cultured prostate stromal cells [328]. Transgenic mice with disrupted A$_{2A}$ receptors have reduced nerve-mediated contractions of the prostate [155], suggesting that prejunctional A$_{2A}$ receptors facilitate sympathetic neurotransmitter release.

Purinergic drugs have been considered for the treatment of benign prostatic hyperplasia [15]. Injection of botulinum toxin, which is known to reduce release of ATP as well as acetylcholine [249], into the prostate have been used to treat bladder obstruction hyperactivity, since it decreases prostate size and thereby improves urine flow rate [82].

Multiple P1 and P2 receptors are expressed by prostate cancer cells [78,180,363,428]. For a detailed coverage of the involvement of purinergic signalling in malignant prostatic hyperplasia see [62].

Sperm

ATP is obligatory for sperm movement. Sperm develop their motile capacity during maturation in the epididymis, during which sperm exonemes change their sensitivity to ATP [447]. Measurement of the ATP concentration in semen has been used to estimate the motility and energy status of human spermatozoa and has been recommended as a method to define optimal conditions for sperm function [325,380], although other groups concluded that ATP measurement had only limited value in the evaluation of semen quality [267,273]. Deficient generation of ATP may cause low sperm motility in some, but not all, conditions of sperm infertility [242]. In P2X1 receptor knockout mice, there was diminished fertility with decreased numbers of spermatozoa in the ejaculate [294].

Changes in ATP content of sperm depends on temperature [71,379]. Spermatozoal ATP concentrations decrease during passage through the epididymis [129]. High flagellar beat efficiency that occurs during hyperactivation is related to a fall in intracellular ATP levels [182]. ATP-supplemented medium has been used for the conservation of human semen for use in artificial insemination [91]. ATP concentration and ATP/ADP ratios in human sperm appear to be unrelated to fertility [419]. Reactive oxygen species, which inhibit sperm motility, is associated with loss of intracellular ATP and motility of spermatozoa ceased when the concentration of ATP was reduced by 85±5 % [88]. Prostasomes transmit signalling complexes between acinar epithelial cells of the prostate and sperm cells and have the capacity for ATP formation [349].

Evidence was presented that an ATP receptor in the egg membrane may be the recipient target for ATP released from sperm, where ATP-induced increase in sodium permeability mediates the initial sperm and egg signal in the fertilisation process [218]. Guanethidine-induced sympathectomy delayed epididymal transit, but did not produce qualitative changes in sperm [195].

In the trout, extracellular ATP and adenosine were shown to influence the in vitro proliferation of spermatogonia and it was suggested that they may be released from nerves to influence the induction, speeding up, then slowing down of spermatogenesis [243]. In humans, extracellular ATP induces significant increase of sperm fertilising potential and the use of ATP for in vitro treatment of spermatozoa during in vitro fertilisation (IVF) for male factor infertility was proposed [350]. Preincubation of fresh spermatozoa with adenosine before the transient co-incubation IVF can also improve the monospermy rate in pigs [137]. Freeze-thawing spontaneously

activated sperm motility did not negatively affect fertility in the carp [48]. The post-thaw motility of avian sperm was improved with a combination of ATP and dimethylacetamide [42]. Cryopreserved sperm penetrated rat oocytes when the gametes were cultured in an ATP- and dibutyryl cAMP-containing medium and the resultant embryos formed blastocysts [445].

While early papers considered the role of ATP in terms of its role largely as an energy source for mitochondrial respiration in sperm, from the early 1990s the possible roles of ATP and its breakdown product, adenosine, were also considered as extracellular signalling molecules. For example, the stimulatory affect of adenosine by regulating adenylate cyclase activity in the fertilising ability of mouse sperm in the early stages of capacitation, i.e., the physiological changes needed for sperm to penetrate and fertilise an egg [164,386]. Ecto-5′ nucleotidase was abundantly detected in the corpora lutea of the ovaries; it showed changes during the oestrous cycle, being maximum coinciding with female sexual receptivity. It was speculated that the high levels of adenosine generated at that time might contribute to sperm capacitation, thus significantly influencing fertility [7]. Adenosine was shown to act via A_2 receptors and adenosine stimulates sperm motility via A_2 receptors [119,132] and to stimulate human sperm motility via A_2 receptors [134,366]. In a later study A_1 receptors were also identified on epididymal bovine spermatozoa [277–279]. A_1 receptor agonists induce calcium release and transient $InsP_3$ increase, capacitance and increase in acrosome reaction rate in human sperm cells [10]. A significant reduction in the number of pups produced by A_1 receptor knockout mice suggests that A_1 receptors must be fully operative to accomplish the optimal degree of capacitation and thereby fertilisation [280] involving modulation of classical Ca^{2+}-dependent PKC isoforms and upregulation of the ERK1/2 phosphorylation [281]. In the hamster, an influx of calcium accompanies capacitation [439] and this may be induced by extracellular ATP. Extracellular ATP was shown to be a trigger for the acrosome reaction in human spermatozoa, via P2 receptors but the subtype involved was not clear at that time [125]. In a later paper from this group it was suggested that a P2X receptor was involved that mediated transient Na^+ influx [127]. Later it was claimed that only a limited population of human spermatozoa has the potential to undergo the acrosome reaction stimulated by ATP and progesterone [401]. Another study suggested that ATP induces acrosomal exocytosis via a P2Y receptor. This leads to an elevation of $[Ca^{2+}]_i$ and to activation of $PKC\alpha$, which phosphorylates proteins, participating in the cascade leading to the sperm acrosome reaction [247].

Guanine, guanosine, inosine and adenosine were found in large amounts in human seminal plasma and more significantly higher in the seminal plasma of oligozoo- and azoospermic men [109]. Guanosine triphosphate had earlier been shown to

regulate sperm adenylate cyclase activity [164]. Both fertilising promoting peptide and adenosine stimulate capacitation and inhibit spontaneous acrosome loss in epididymal mouse spermatozoa [138].

A detailed study of the expression of P2X receptor subtypes on the various germ cell types throughout the different stages of the cycle in the seminiferous epithelium within which spermatogenesis takes place from sperm spermatogonia through spermatocytes to spermatids [145]. P2X2 and P2X3 receptors were always observed together on the same cells at the same stages; P2X7 receptor expression was present throughout all stages, P2X5 receptors were expressed in the later stages. It was suggested that purinergic signalling may play a role in the maturation of germ cells at the different developmental ages that exist alongside each other in the adult testis (see Table 1). P2X1, P2X2, P2X3 and P2X4 receptors were localised in the head, probably on the acrosome, of immature sperm in human, mouse and hamster caput epididymis, but this staining was reduced progressively through the epididymis and was absent on mature sperm in the cauda epididymis, except in humans where P2X4 receptors were retained in the cauda epididymis [24] These changes in localisation of P2X receptors were coincident with the maturational changes seen in sperm as they travel through the epididymis, suggesting a role for purinergic signalling in sperm maturation and possibly fertility. P2X2 receptors have been identified on mouse epididymal sperm, localised to the sperm midpiece [241]. ATP-induced current on mouse spermatozoa was mediated by P2X2 receptors; however, despite the loss of ATP-gated current, spermatozoa from P2X2 knockout mice had normal progressive motility, hyperactivated motility and acrosome reactions [300]. Nevertheless, fertility of male P2X2 knockout mice declined with frequent mating over days, suggesting that P2X2 receptors add a selective advantage under these conditions.

Sperm preincubation with oestradiol $17\beta E_2$ inhibited the effects of extracellular ATP on sperm plasma membrane potential variations and the acrosome reaction [352]. Extracellular ATP improves human sperm motility parameters and provides a rational explanation for increased IVF percentages when sperm is treated with ATP [103]. The effects of ATP on acrosomal exocytosis, protein tyrosine phosphorylation and sperm maturity parameters were quantified. While ATP did not affect acrosomal exocytosis or protein tyrosine phosphorylation in sperm from healthy donors, it significantly altered several motility parameters, with the largest effect manifested in increased curvilinear velocity and percentage hyperactivation. ATP similarly affected sperm selected for poor motility and thawed cryopreserved sperm, although to a lesser extent on sperm with normal motility. These important findings constitute a novel therapeutic modality in the treatment of male infertility. Extracellular ATP was shown to

Table 1 Summary of P2X-immunopositive cells in the seminiferous tubules throughout the l4 stages of the seminiferous epithelium

Cell-types	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
A	P2X2 / P2X3 (I–VIII)													
B				P2X2 / P2X3 (IV–VIII)										
PL						P2X2 / P2X3 (VI–VIII)								
P	P2X2 / P2X3 (I–VIII)									P2X5 (X–XI)				
Di													X5	

Sperm	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Sperm	P2X2 / P2X3 (1–8)									P2X5 (10–13)				

Sperm	15	16a	16b	17a	17b	18	19a/b	8
		P2X2 / P2X3 (16a–19a/b)						X2 / X3 / 19c / X7

Serto	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
	P2X2 / P2X3 (I–VIII)													
	P2X7 (I–XIV)													

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The stages of the cycle of the seminiferous epithelium are given in *roman numerals*. *Shaded boxes* indicate the presence of immunopositive cells for a single P2X receptor subtype throughout the respective stages of the cycle. *Italic numerals* indicate the developmental steps of spermatid maturation. Throughout stages I to VIII younger (1–8) and older (16a–19c) generations of spermatids coexist, whereas through stages IX to XIV only one generation of developing spermatids is present

X2 P2X2 receptor, *X3* P2X3 receptor, *X5* P2X5 receptor, *X7* P2X7 receptor, *A* type A spermatogonia, *B* type B spermatogonia, *P* pachytene spermatocytes, *Di* diplotene spermatocytes, *PL* preleptotene spermatocytes, *Sperm* spermatids, *Serto* Sertoli cells

similarly alter motility and improve the fertilising capacity of mouse sperm [344].

Spermatozoa possess a high level of ecto-ATPase activity [256]. The effect of ATP and adenosine on sperm function could be regulated by hydrolysis of ATP, ADP and AMP by ecto-NTPDases and ecto-5′-nucleotidases identified in human spermatozoa [346].

In asthenozoospermic or oligoasthenozoospermic patients with less than 1,000,000 round spermatid cells/ml, the concentration of ATP is significantly lower than normal [70]. Semen ATP content was not helpful in predicting the occurrence of pregnancy in which the female partner was normal and the male partner had sperm concentrations of 720×10^6/ml [442]. The enzymatic treatment of spermatozoa with a trypsin solution enhanced ATP concentration and improved motility [121]. Control of anion and fluid secretion by ATP released from sperm during transit was acting via P2 receptors [441].

In summary it appears that ATP is involved in sperm motility and fertilising ability by two independent mechanisms: on the presence of high intracellular levels of ATP to supply the energy for mitochondrial respiration; and release of ATP from sperm during transit to regulate their microenvironment by acting on receptors for both ATP (probably via $P2Y_1$ receptors) and its breakdown product adenosine (via A_1 receptors) to activate the acrosome reaction to facilitate motility and fertilisation. Treatment of sperm with ATP used for IVF is now being explored. For example, a recent paper showed, with IVF experiments in mice, that treatment of sperm with ATP improved the in vitro fertility rate of genetically modified (transgenic and knockout) mice with low fertility [411].

Female reproductive organs

Purinergic signalling has been identified in the ovary, oviduct, amnion, oocytes, uterus and cervix, placenta and umbilical vein, vagina and mammary glands.

Ovary

The principal source of estrogens and androgens in the female are ovarian granulosa and theca cells.

A surge of pituitary LH 12 h prior to ovulation stimulates oocyte maturation. In rabbit isolated ovarian follicles ATP inhibited LH-stimulated testosterone accumulation [245]. A 7-fold amplification of LH-stimulated cAMP accumulation and progesterone secretion in rat luteal cells was produced by adenosine, but it did not have a similar effect on LH-stimulated cAMP accumulation and androgen secretion in Leydig cells [159]. Adenosine had predominantly inhibitory actions on hormone-induced granulosa cell differentiation [210]. Adenosine acting via A_2 receptors stimulated adenylate cyclase in rat ovarian membrane preparations and preovulatory granulosa cells [37]. Progesterone secretion by rat granulosa cells was regulated by AMP-activated protein-kinase [403]. Adenosine and prostaglandin $F_{2\alpha}$ are possible regulators of luteal cell function, acting by local control of the action of LH [31]. This group later showed that there was no effect of adenosine on androgen secretion in Leydig cells. Rather, adenosine produced marked amplification of FSH-stimulated cAMP accumulation and steroid secretion from granulosa cells from both rat and human ovaries [32,323]. Luteal cell ATP is rapidly depleted by LH. This appears to be a physiological event as a result of cells undergoing apoptosis (i.e., luteolysis) at the end of the pseudopregnant cycle [382].

Gonadotrophins as well as adenosine can affect ATP levels in granulosa cells [36]. The effect of purine nucleotides on human and porcine granulosa cells was examined using luteinised cells [184]. The authors suggested that the results indicated the presence of P_{2U} (i.e., $P2Y_2$ and/or $P2Y_4$) receptors on human granulosa cells and P_{2U} and P_{2T} (i.e., $P2Y_{12}$) receptors on porcine granulosa cells, but the functional roles of these receptors was unclear. P_{2U} receptor mRNA was found in human granulosa cells and ATP/UTP was shown to cause rapid transient increase in $[Ca^{2+}]_i$ [391]. ATP has an antigonadotrophic action in human granulosa cells [392]. This group later showed that ATP induced nuclear translocation of phosphorylated ERKs and the induction of egr-1 and c-raf-i expression in the human ovary [393]. This suggested that the mitogen-activated protein kinase signalling pathway plays a role in mediating the effects of ATP on gonadotrophin-induced progesterone secretion in the human ovary. P2, but not P1, receptors were shown to be expressed on chicken granulosa cells [292]. Calcium oscillations were mediated by $P2Y_2$ and/or $P2Y_4$ receptors in human granulosa-luteal cells [228,385].

ATP acts on granulosa cells via a mechanism that involves $P2Y_2$ receptor stimulation and the participation of ryanodine receptors [288]. UTP-sensitive P2Y receptors are expressed in cultured murine theca/interstitial cells and occupation of these receptors leads to the activation of mitogenic signalling

pathways to promote cell proliferation [413]. The authors concluded that regulation of proliferation of these cells and steroidogenesis plays an important role in ovarian pathophysiology, since theca hyperplasia is found in rats with polycystic ovarian syndrome [357]. Signalling to ovarian perifollicular smooth muscle changes from mediation via P2X2 receptors to P2X1 receptors during pregnancy, and there is an increase in P2X2 receptor expression on ovarian vascular smooth muscle [191].

Menopause is associated with a decline in ovarian function. In a murine menopause model, ovarian levels of P2X2 receptor protein increased with ageing, suggesting that the P2X2 receptor is involved with menopause/ageing-related decline in ovarian function [464].

Thecal cells, which form the external layer surrounding the ovarian follicle, are involved in the synthesis of androgens. Apoptotic cell death of porcine ovarian theca cells is caused by ATP acting via P2X7 receptors [412].

The cat ovary is densely innervated by sympathetic nerves, which release NA and ATP, as well as by intrinsic neurons, most of which are parasympathetic. The storage and release of NA and ATP decreased after ovulation, but sympathetic nerve activity appears to increase during ovulation [222]. Sympathetic neurotransmitters are present in the rat ovary during early postnatal development and affect steroidogenesis before the ovary becomes responsive to gonadotrophins and before the first primordial follicles are formed.

Chinese hamster ovary (CHO) cells contain very few endogenous receptors, which makes them ideal transfection recipients. However, a constitutive ATP receptor linked to arachidonic acid release has been reported [116]. A two-phase response of acid extrusion triggered by $P2Y_2$ purinoceptors on CHO cells was later reported [310]. This ATP receptor is also linked to the mobilisation of $[Ca^{2+}]_i$ and through pharmacological characterisation it appears to be a $P2Y_2$ and/or $P2Y_4$ receptor [178]. Exposure to ATP or UTP on the apical side of confluent CHO cell monolayers produced an increase in $[Ca^{2+}]_i$ [407]. It was also shown that functional expression of human CFTR in the plasma membrane of CHO cells infected with adenovirus regulated the increase in intracellular Ca^{2+} produced by ATP released from the cells. A_{2B} receptors have also been identified on CHO cells [6].

The hydrolysis of ATP and ADP is decreased by ovariectomy and oestradiol replacement therapy [322]. Ovarian tumours arise mainly from the surface of simple squamous-to-cuboid mesothelium that covers the ovary. Mitogen-activated kinase in pre-neoplastic and neoplastic surface epithelial cells is stimulated by ATP, suggesting that co-released ATP from sympathetic nerves may play a role in regulating cell proliferation in both normal and neoplastic ovarian surface epithelial cells [80]. FSH has a stimulatory effect on ATP release and platelet aggregation [25]. Phosphodiesterase 8 has been found

in the mammalian ovarian follicle and may be involved in hormonal regulation of folliculogenesis [359].

Oocytes

K^+ current responses to FSH or adenosine in monolayers of small follicular cells surrounding a single large oocyte of *Xenopus* are suppressed by ATP [136]. P2 receptors are expressed by the follicular cells of *Xenopus* [207,286]. Since UTP and ATP are equipotent, this effect could be mediated by a $P2Y_2$ or $P2Y_4$ receptor subtype [136]. Moreover, it has been suggested that there are close interactions between A_2 and P2Y receptors in *Xenopus* follicles [19]. Cultured CHO cells express a P2 receptor equi-reactive to ATP and UTP [178], probably the $P2Y_2$ and/or $P2Y_4$ subtype. Extracellular ATP has been shown to facilitate the development of parthenogenetically activated bovine oocytes [444]. Redistribution of mitochondria leads to bursts of ATP production during spontaneous mouse oocyte maturation [453]. The *Xenopus* oocyte can release ATP, both in basal conditions and in response to mechanical stimulation or osmotic changes to have paracrine actions [5,46,160,259].

P1 receptors on follicular cells mediate K^+ current responses, while $P2Y_2$ receptors are involved in the activation of Cl^- currents [356]. These authors and others [329] suggested that these effects are consistent with intrafollicular ATP signalling that modulates oocyte maturation.

In *Xenopus* oocytes ectonucleotidases rapidly and locally convert ATP to adenosine, which leads to activation of A_{2B} receptors [264]. A study in human ovaries has shown a relationship between the higher follicular fluid adenosine levels and follicular/oocyte maturity [433]. The ATP content and fertilisation outcome of bovine oocytes were shown to increase with maternal age [179].

Cumulus cells consist of a cluster of follicle cells that surround a freshly ovulated ovum and they are dispersed at fertilisation by the contents of the acrosome. Data has shown that cumulus cells express $P2Y_2$ and possibly $P2Y_{12}$ receptor subtypes, but not P2X receptors (Bains, unpublished data).

Fallopian (uterine) tubes/oviduct

Released oocytes are captured by the fallopian tubes/oviduct and transported in an appropriate condition to the site of fertilisation in the distal ampullary region of the tube. They also provide tubal secretions for sperm to migrate proximally from the uterus. The sperm acrosomal reaction occurs mainly at the cervix and uterus. Purinergic receptor-mediated increases in $[Ca^{2+}]_i$ were described in single isolated epithelial cells of the human uterine tube [92,384]. P2X1 and P2X2 receptors are expressed on blood vessels in rat fallopian tubes, but not peritubular smooth muscle [28]. ATP-mediated contraction of the human fallopian tube is enhanced with acute

purulent inflammation, probably by upregulation of P2X1 and P2X2 receptors [463]. A functional purinergic receptor was identified on primary cultures of bovine oviduct epithelia [84,420]. ATP evoked a rapid increase in $[Ca^{2+}]_i$ in oviductal endosalpingeal cells isolated from heifers at different reproductive stages [397]. The receptor involved in increases of $[Ca^{2+}]_i$ and regulation of ion transport on the basal surface of bovine oviduct cells is likely to be the $P2Y_2$ or $P2Y_4$ subtype since UTP and ATP were equipotent [85]. Both ATP and adenosine receptors are present on single ciliated oviductal cells acting via $P2Y_2$ an A_{2A} receptor subtypes, respectively [287]. The ciliated cells from oviduct play an important role in the control of mucociliary transport velocity of gametes and embryos. ATP increases ciliary beat frequency [29] and this effect is modulated by adenosine [30]. Purinergic signalling via $P2Y_2$ receptors also constitutes a key mechanism for regulating chloride secretion and thus fluid formation in the bovine oviduct [193]. In a later paper, they claimed that purinergic activation of a calcium-dependent, apamin-sensitive potassium conductance was essential to promote chloride secretion [194].

Uterus

The uterus is the site of sperm migration, implantation of the fertilised ovum, placental development, embryo and foetus development and the generation of contractions at term for delivery. The myometrium and endometrium are the main uterine functional tissue types.

Myometrium

ATP and ADP contract the uterus [100,311] partly through the action of prostaglandins induced by P2Y receptor occupation [4,291,390]. In the longitudinal muscle of the mouse myometrium, ATP produced a biphasic response, an initial hyperpolarisation followed by a depolarisation, which remained the same in both non-pregnant uterus and throughout pregnancy [306]. In the cat uterus reversal of the ATP response during gestation was reported, namely inhibition of spontaneously generated electrical and mechanical activity in the virgin uterus, but excitation in the early stages of gestation [55]. Whole cell voltage clamp studies of cultured smooth muscle cells from the pregnant rat myometrium showed that ATP, but not adenosine, AMP or ADP, activated a monovalent cation-selective and oestrogen-sensitive conductance [167].

Data from experiments using isolated uteri of non-pregnant guinea-pigs suggests that myometrial cells contain a mixture of P2 receptors, a UTP-sensitive receptor (probably $P2Y_2$ or $P2Y_4$), another P2Y receptor and an α,β-meATP-sensitive receptor (probably P2X1, P2X2/3 or P2X3) [321]. Using immunohistochemistry, P2X2 receptors were expressed by the smooth muscle of the rat uterus, with only weak

immunostaining of P2X1 receptors [28]. P2X1 and/or P2X2/3 receptor-mediated contractions of isolated human pregnant uterus were evoked by α,β-meATP and shown to be term-dependent, increasing during pregnancy [462]. P2X1 and P2X2 receptors were identified in uterine smooth muscle, suggesting that P2X1 and P2X2/3 receptors mediate contractions of the pregnant human uterus, although it is likely that P2Y$_2$ and/or P2Y$_4$ receptors are also involved, to account for the high potency of UTP [461].

P2X1 receptors have been shown to be closely associated with connexin 43 (Cx43) in the human myometrium, and may be involved in gap junction formation [183]. Expression of Cx43 is upregulated in the later stages of pregnancy and peaks near parturition, perhaps playing a role in the synchronised and coordinated uterine contractions at parturition. It was suggested that the high levels of ATP released from uterine cells during childbirth may act on P2X1 receptors in gap junctions to inhibit expression of Cx43, thereby reducing gap junctions and coordinated contractions in the post-partum uterus, when P2X1 receptor expression peaks [199]. Enhanced expression of P2X4 and P2X7 receptors has been observed in the myometrium of pregnant rats in preterm delivery models, which could be related to uterine contraction leading to term and preterm delivery [406].

It has been claimed that extracellular ATP is essential for the initiation of contractions and control of their frequency (but not contractile force) and may be involved in the pace-making mechanism for the generation of uterine contractions [173], perhaps via P2Y$_2$ receptors [174]. Neocuproine, a copper chelator, can affect uterine contractile activity by modulation of purinergic excitatory responses [217]. Two different ectonucleotidase phosphohydrolases have been described in the rat myometrium [252]. Changes in apyrase activity were shown to occur during pregnancy [409].

Adenosine acting via A$_1$ receptors contracts the smooth muscle of the virgin guinea-pig uterus [361,372]. It has been suggested that there are two types of receptors for adenosine, the breakdown product of ATP, in the myometrium, one mediating excitatory, the other inhibitory responses [124]. It was later claimed that A$_1$ receptors mediate excitatory responses mainly during the proliferation phase of the menstrual cycle, while A$_2$ receptors, mediating excitation, are largely involved during the secretory phase [358]. Adenosine is also a prejunctional modulator of adrenergic neurotransmission to the human uterus [440]. Evidence for the presence of the A$_{2B}$ subtype of the adenosine (P1) receptor in the rat myometrium has also been presented [144]. A purinergic receptor was identified in human myometrium membranes using 5′-N-[^{3}H]ethylcarboxamide-adenosine as a radioligand [348]. The binding site had the characteristics of the A$_2$ adenosine receptor and some of those of P2 receptors too. Later pharmacological studies suggested that both P1 and P2

receptors are present in the guinea-pig myometrium, both mediating contraction [372].

It has been shown that ATP induced ion currents and contractions via P2X7 receptors in freshly isolated myometrial cells from pregnant rats and that P2X7 receptor mRNA was localised in these cells [282,283], supporting the earlier findings of Urabe et al. [406]. These authors showed further that Mg^{2+} blocked the P2X7 receptor-mediated contraction in tocolysis and suggested that targeting P2X7 receptors could lead to novel treatments for the prevention of uterine contractions in preterm deliveries. The ectonucleotidase, NTPDase 1, has been localised on the surface of myometrial smooth muscle cells and blood vessel endothelium [275].

Endometrium

ATP, released from nerves innervating the uterus and uterine blood vessels and by autocrine or paracrine release from epithelial cells, plays an important role in regulating endometrial functions such as local (paracrine) coordination of sperm migration and capacitation, implantation of the fertilised egg, endometrial fluid formation and composition; cell proliferation and differentiation in post-partum reorganisation; and endometrial epithelial cell differentiation and apoptosis.

Human endometrial epithelial cells express P2X7 receptors [236]. P2X5 and P2X7 receptors were shown to be immuno-reactive in the rat uterine epithelium [28]. Changes in the expression of P2X receptor subtypes in uterine epithelial cells were shown during the endometrial cycle and early pregnancy in the rat [368] and in humans [150]. These changes are related to, and mediate differentiation and apoptosis of the endometrium.

One of the functions of ATP is regulation of the steroid-binding activities of oestradiol receptors [220]. Extracellular nucleotides may play an important role in the fine-tuning of the uterine fluid microenvironment by regulating both Cl$^-$ secretion and Na$^+$ absorption across the endometrium [92,425], since over-expression of Na$^+$ channels in mouse endometrial epithelium suppresses ATP-induced Cl$^-$ secretion [426]. Oestrogen and progesterone differentially regulate UTP-stimulated anion secretion in endometrial epithelial cells by altering the expression of P2Y$_2$ receptors and basolateral K$^+$ channels [314].

Extracellular ATP activates nuclear translocation of ERK1/2 leading to the induction of matrix metalloproteinase expression in human endometrial stromal cells [76]. In a later paper, these authors showed that the P2Y$_2$ receptor is expressed by stromal cells, suggesting that it mediates the expression of the early growth response 1 and inhibits stromal cell viability [77]. The distribution of ecto-nucleotidases in human cyclic and post-menopausic endometrium has been described [8]. Ecto-NPP3 was identified as a new biological marker of tubal metaplasia. It has been proposed that uridine diphosphate-

glucose and its $P2Y_{14}$ receptor are key players able to trigger innate uterine mucosal immunity in human endometrial epithelial cells by inducing interleukin-8 [18].

In the rat, P2X7 receptors are localised on eosinophils, macrophages and fibroblasts of the endometrium during oestrus [216]. The authors speculated that ATP-mediated responses may be important in uterine preparation and remodelling before implantation, and that the presence of P2X7 receptors in stromal cells may indicate their involvement in immune and inflammatory responses. There is a strong transitory expression of A_{2B} adenosine receptors on the mouse uterus after blastocyst implantation during early post-implantation development [40].

In the rat uterine epithelium on day 1 of pregnancy, there was no expression of P2X7, $P2Y_2$ or $P2Y_4$ receptors, although there was some diffuse immunolabelling of $P2Y_1$ receptors. On day 3, P2X7 and $P2Y_2$ receptors were present and confined to the lateral plasma membrane of the epithelium. At the time of implantation on day 6, there was strong labelling for P2X7 and $P2Y_2$ receptors, found along the entire surface of the apical epithelium, suggesting a role for calcium-modified events proceeding and facilitating attachment and implantation of the blastocyst [370].

In the pig uterus, epithelial cells of the endometrial gland expressed $P2Y_2$ and $P2Y_4$ receptors [313]. P2X7 receptors were shown to be expressed on the luminal surface of endometrial cells prior to implantation in rats [396] and in the periovulatory period in humans [150]. In the rat, at the time of implantation on day 6, apoptosis was reduced in the non-implantation uterine epithelium, but was markedly increased adjacent to the implanting blastocyst [396]. It was proposed that P2X7 receptor-mediated apoptotic cell death is an important regulatory factor involved in uterine remodelling prior to and during implantation. It was later proposed that P2X7 receptor-mediated apoptotic cell death and endometrial remodelling facilitate the attachment and implantation of the blastocyst to the endometrium [233].

In the human endometrium, as in the cervix and vagina, P2X7 receptors regulate terminal differentiation and are the main physiological pro-apoptotic mechanism in vivo [150]. Activation of the P2X7 receptor induces pore formation in the plasma membrane and facilitates terminal differentiation and apoptosis [117]. In the human endometrium in vivo, expression of the P2X7 receptor depends on the state of tissue differentiation. In the early proliferative phase, the receptor is diffusely expressed in the plasma membrane, while in more advanced stages of maturation, i.e., mid-late proliferative phases, expression of the P2X7 receptor increases in regions of the apical (luminal) plasma membrane (Fig. 5). Treatment of cultured epithelial cells with ATP or $2'(3')$-O-(4-benzoylbenzoyl) adenosine $5'$-triphosphate (BzATP) induces pore formation preferentially in the apical membrane, suggesting that the terminal differentiation in vivo is mediated by

formation of P2X7 receptor pores, and that the apical localisation of P2X7 receptors in the endometrium in vivo reflects the accumulation of P2X7 receptor aggregates within apical P2X7 receptor pores. The apical localisation of P2X7 receptor pores in vivo is not specific to the endometrium, and is found also in other normal human monolayered epithelia.

Dysregulation of the P2X7 proapoptotic receptor plays a role in the development of endometrial cancers, as well as in other types of cancers of epithelia derived from the ectoderm, the urogenital sinus and the distal paramesonephric duct [236]. Decreased levels of P2X7 receptors are associated with cancer development, and in women decreased expression of the P2X7 receptor can be found in pre-cancerous endometrial lesions [237]. Mechanisms that could lead to decreased expression of the P2X7 receptor include epigenetic dysregulation through hypermethylation of the P2X7 receptor gene [457]; enhanced degradation of the P2X7 transcript [456]; and decreased glycosylation [117]. Of the naturally occurring P2X7 splice variants, the P2X7j receptor, a dominant negative form that blocks P2X7 receptor-mediated actions [118], is co-expressed with the P2X7 receptor in epithelia, primarily those of the female reproductive tract. The P2X7j isoform can hetero-oligomerise in the plasma membrane with the P2X7 receptor, and form non-functional complexes. Co-expression of P2X7 receptors plus P2X7j receptors blocks ATP-induced pore formation, and abolishes baseline and agonist-induced apoptosis.

Cervix

The cervix is the distal part of the uterus, which opens into the vagina. Columnar (endocervical) epithelium lines the proximal region of the cervix bordering the endometrial epithelium, while stratified squamous epithelium lines the distal part of the cervix, the ectocervix, which is continuous with the vaginal epithelium. A major function of the cervical epithelium is the synthesis and secretion of cervical mucous. The cervical mucous controls access to the uterine cavity for microorganisms and particulate matter. The content and composition of the cervical mucous change throughout the menstrual or oestrous cycles, and is influenced by changes in levels of ovarian oestrogen, androgens and progesterone [153]. During the pre-ovulatory phase, changes in mucous facilitate sperm movement towards the fallopian tube, and provide an optimal milieu for sperm capacitation. During other phases of the cervical cycle, changes in mucous characteristics impede sperm penetration and capacitation.

Cervical cells express different subtypes of purinoceptors. HeLa cervical epithelial cells express $P2Y_1$, $P2Y_2$, $P2Y_6$ and P2X7 receptors [429]. In the rat cervix, expression of P2X3 receptors on afferent nerves increases during pregnancy, and this increase is probably related to coordination of cervical tone and contraction/dilation during labour [317]. Primary cultures of human endocervical and ectocervical epithelial
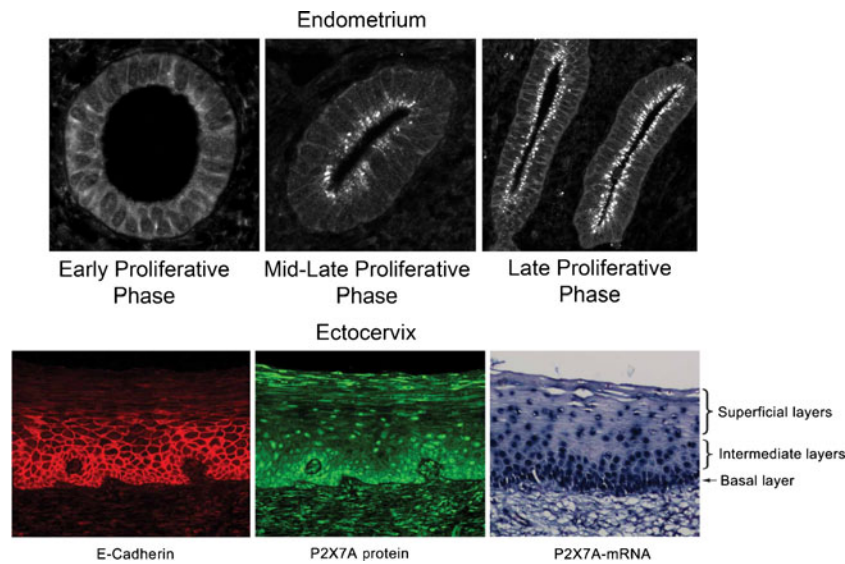
Fig. 5 *Upper panel* (endometrium): P2X7 receptor expression in cross sections of human endometrial glands at different phases of the menstrual cycle. The increases in P2X7 receptor immunoreactivity in apical regions of the plasma membranes (facing the lumen) represent P2X7 aggregates within pores. (Reproduced from [237], with permission from Springer.) *Lower panel*: full-length P2X7 receptor type-A (P2X7A) expression in cross sections of human ectocervix. P2X7A receptor immunoreactivity in reserve cells (basal layer) represents de novo-synthesized P2X7A molecules. P2X7A receptor immunoreactivity in intermediate layers represents mainly P2X7A molecules aggregating into pores. P2X7A immunoreactivity in superficial layers represents P2X7A aggregates within pores that are retained in clumps of squames. (Reproduced from [150], with permission from Wiley)

cells express P2Y$_2$ and P2X4 receptors, which are involved in epithelial transport [148] as well as P2X7 receptors, which are involved in the regulation of terminal differentiation and apoptosis of endocervical and ectocervical epithelial cells. The P2X7 receptor may also be involved in the control of cervical infections, since P2X7 receptor-mediated activation of cervical epithelial cells inhibits infection by *Chlamydia* and mycobacteria [87].

Data from experiments using three-dimensional culture models of human normal epithelial cervical cells suggest that the purinergic system controls cervical mucous secretion in vivo. Activation of the P2Y$_2$ receptor stimulates [Ca^{2+}]$_i$-dependent chloride secretion, osmotic water efflux and increased transepithelial fluid secretion [146]. Activation of the P2X4 receptor, induces a slower and longer increase in tight junctional resistance and decreased transepithelial fluid secretion [147]. The P2X4 receptor effect is mediated by [Ca^{2+}]$_i$-dependent activation of phospholipase (PL)-D, upregulation of diacylglycerol and activation of PKC-mediated threonine dephosphorylation of the tight-junctional protein, occludin [153,458]. Both P2Y$_2$ and P2X4 receptors are located on the apical (luminal) surface of the epithelial cells [146,147,151] (Fig. 6).

The dual regulation of transepithelial fluid secretion suggests that net fluid secretion (and thus cervical mucous secretion) in vivo is determined by the combined actions of the P2Y$_2$ and P2X4 receptors. It was also suggested that P2X4 receptor-induced decrease in permeability and a decrease in mucous secretion in the micro-environment of the endocervix

in vivo could result in retention of sperm cells in endocervical crypts [152]. Longer retention and longer exposure to endocervical fluid can improve capacitation and provide for graded transport of sperm cells to the uterus and tubes [177].

In the mammalian male reproductive tract, the epididymal and vas deferens epithelial cells acidify the lumen via an apical V-H$^+$-ATPase mechanism; the epididymal and vas deferens V-H$^+$-ATPase transporter is regulated by the P2X4 receptor [52]. A similar mechanism is present in the cervix and vagina, where baseline active proton secretion occurs constitutively throughout life, and the acidification is upregulated by oestrogen ([154]; see [133]).

Endocervical and ectocervical epithelial cells express the P2X7 receptors and the main role of P2X7 receptors in these epithelia is regulation of terminal differentiation and control of apoptosis [236,237]. Expression and function of the P2X7 receptor in the monolayered endocervical epithelium are similar to those in the endometrium, and decreased expression of the P2X7 receptor is associated with the development of endocervical cancer.

In the ectocervix, i.e., the distal part of the cervix which projects into the vagina, the P2X7 receptor is expressed by the stratifying ectocervical epithelial cells. In vivo, there is an abundance of P2X7 receptor expression by reserve cells in the basal layer of the epithelium and an abundance of expression of P2X7 receptor *aggregates* in more superficial cells undergoing terminal differentiation in vivo [150]. Data from cultured ectocervical cells showed preferential apical localisation of P2X7 receptors and preferential P2X7 receptor
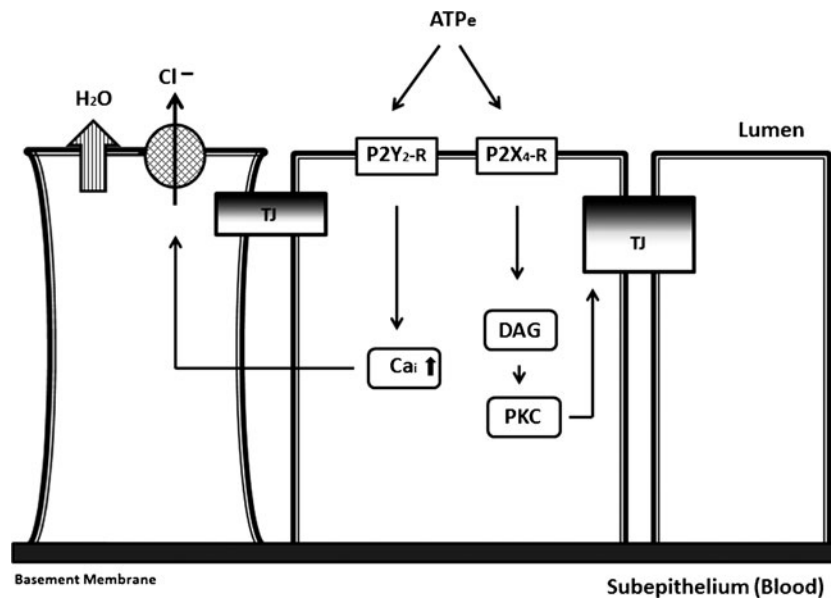
Fig. 6 P2Y$_2$ and P2X4 receptor regulation of epithelial permeability and transepithelial transport across human cervical cultures (compiled from data in [146,147,152,153,458]). Extracellular ATP (ATP$_e$) modulates paracellular permeability by activating two distinct apically localized receptor–effector mechanisms. The P2Y$_2$ receptor stimulates acute release of calcium from internal stores, active Cl$^-$ secretion and osmotic water efflux, resulting in cell shrinkage and reciprocal increase in the pericellular intercellular space volume; this leads to decreased paracellular resistance and an increase in the paracellular permeability. P2X4 receptors stimulate sustained release of diacylglycerol (DAG) and activation of protein kinase C (PKC). PKC modulates the tight junctional resistance by dephosphorylation of the tight junctional protein occluding, and stimulates a slow and long increase in the tight junctional resistance and a reciprocal decrease in paracellular permeability. The net effect on epithelial permeability is dependent on the spatial and temporal effects of the P2Y$_2$ and P2X4 mechanisms. (Schematic courtesy of G.I. Gorodeski)

pore formation in the apical membrane. Similar data were found also in the epidermis [135], suggesting that P2X7 receptor pore formation initiates terminal differentiation in stratifying epithelia in vivo. Decreased levels of the P2X7 receptor are associated with the development of ectocervical cancers, and in women decreased expression of the P2X7 receptor can be found already in pre-cancerous lesions [236,237]. In cultured human ectocervical cells treatment with ATP or BzATP augments apoptosis [118], and in mice local application of BzATP on the skin augments apoptosis mainly of epidermal reserve cells, and blocks carcinogen-induced skin cancer [135]. The authors suggested that activation of P2X7 receptor-dependent apoptosis could be a novel chemotherapeutic growth-preventive modality for pre-cancerous and early cancerous epithelial lesions [135,149].

Amnion

The amniotic membrane forms the sac which contains the embryo, and it connects to the embryo at the umbilical cord. ATP activates the PLC cascade system in human amnion cells without increasing prostaglandin production [410].

Placenta

The placenta and umbilical vessels are involved in regulation of blood flow and control of transport of materno–foetal fluid and solutes, as well as in steroidogenesis. High plasma circulating levels of adenosine have been measured at birth in sheep and it was suggested that the adenosine, probably derived from the placenta, inhibits thermogenesis before birth. After cord occlusion, adenosine decreases, allowing thermogenesis to begin. These data suggest that adenosine plays a role in regulating foetal metabolism [360]. Increases in plasma levels of adenosine were also found in pregnant women, and the authors suggested that the adenosine is derived, in part, from platelet activation and an increase in 5′-nucleotidase activity during pregnancy [450]. Hyperemesis gravidorum is characterised by an overaction of sympathetic nerves and enhanced production of TNF-α. Plasma adenosine is significantly increased in hyperemesis gravidarum, and could serve as a prejunctional modulator of sympathetic neurotransmission and to counteract further progression of the disease [451].

Chronic caffeine or theophylline intake during pregnancy inhibits A$_1$ receptor function in both maternal and foetal brains [230]. Adenosine is important as a metabolite of ATP in the human term placenta in ischaemic or hypoxic conditions [254]. Binding sites were identified in human placenta with characteristics of an adenosine receptor, probably the A$_2$ receptor subtype [130]. Adenosine uptake by human placenta has been described, including its metabolism both intra- and extracellularly [2]. Adenosine uptake at both the maternal and foetal sides of the syncytiotrophoblast was demonstrated, and shown to be inhibited by dipyridamole; this suggested that the

placenta is involved in the regulation of adenosine-related function [438]. Adenosine induced relaxations in segments of human chorionic arteries and veins pre-contracted with K^+ and pharmacological analysis led to the conclusion that both A_1 and A_2 receptor subtypes were involved [339]. Adenosine causes a biphasic response (vasoconstriction followed by vasodilation) in the ovine foetal placental vasculature [337]. Chromosomal abnormality exhibited in TR21 (Down syndrome) pregnancies often end in abortion and represents a good model for examining the mechanism regulating miscarriage. Downregulation of A_1 and A_{2B} receptors in human trisomy 21 mesenchymal cells from first-trimester chorionic villi has been reported [142].

ATP (and adenosine) caused vasoconstriction of the human foetoplacental vascular bed, but there was reduced sensitivity to adenosine in cotyledons from some placentae that may represent downregulation of adenosine receptors as a result of exposure to adenosine during delivery [253]. ATP, α,β-meATP and β,γ-methylene ATP constricted the perfused human placental cotyledon via P2X receptors, but vasodilation via P2Y receptor-mediated NO release from endothelial cells was also demonstrated [94,336]. ATP increased Ca^{2+} uptake by microvillous boarder vesicles formed from term human placental syncytiotrophoblasts [312]. Continuous measurement of ATP was examined by ^{31}P-nuclear magnetic resonance spectroscopy in human perfused placenta in vitro during ischaemia [257]. It was shown that after 4 h of ischaemia, ATP decreased by 85 % of controls, but after reperfusion ATP levels increased. Stimulated increase in $[Ca^{2+}]_i$ by ATP and UTP in human term placental calls [319] suggests that a $P2Y_2$ and/or $P2Y_4$ receptor is involved.

In a study of P2 receptor subtypes in the arterial vascular bed of human perfused placental cotyledons [334], it was shown that:

(1) The contractile responses mediated by α,β-meATP and ATP of the placental smooth muscle was resistant to desensitisation and insensitive to antagonism by PPADS, thus showing dissimilarity to the classic P2X1 receptor.
(2) A smooth muscle $P2Y_2$ or $P2Y_4$ receptor also mediates vasoconstriction to UTP and ATP.
(3) Vasodilation responses are mediated by $P2Y_1$ receptors located on endothelial cells that, when occupied by ADP, lead to release of NO.

Evidence has been presented that a similar, novel P2X receptor is present in human placental chorionic surface arteries [95]. mRNAs for P2X1, P2X4, P2X5, P2X6 and P2X7 receptors were identified in the vascular smooth muscle from chorionic (and umbilical) arteries and veins, while mRNA for $P2Y_1$, $P2Y_2$ and $P2Y_6$ receptors was found on the endothelium of these vessels [170] highlighting the role of extracellular

nucleotides as regulators of foetal blood flow. Adenosine, acting via A_{2B} receptors, has also been claimed to produce human chorionic vasoconstriction in perfused cotyledons and that this involves synthesis of a thromboxane receptor activator or a related prostanoid [98]. In a study of P2 receptor mRNA and protein expression in human placental villus fragments at two stages of gestation, the first trimester and term [342], P2X4, P2X7 and $P2Y_2$ (but not $P2Y_4$) receptor subtypes were found and fluorescent calcium imaging experiments showed that ATP, UTP and $2'(3')-O-(4$-benzoylbenzoyl)adenosine 5'-triphosphate caused significant elevation of $[Ca^{2+}]_i$ in term compared to the first trimester. Systematic pharmacological studies of purinergic signalling in human placenta showed functionally active P2X4, P2X7, $P2Y_2$ and $P2Y_6$ receptors on cytotrophoblast cells and it was proposed that activation of these receptors and subsequent elevation of $[Ca^{2+}]_i$ modulates syncytiotrophoblast homeostasis and maternofoetal ion exchange [341]. $P2Y_1$ and $P2Y_2$ receptors are unevenly distributed along the human placental vascular tree; there is a 6- to 8-fold increase in these receptors from the cord to the chorionic or cotyledon vessels [69]. In the cord and chorionic vessels the receptors are mainly on smooth muscle, while in the cotyledon vessels $P2Y_1$ and $P2Y_2$ receptors were equally distributed between endothelial and smooth muscle cells. mRNA for $P2Y_4$, $P2Y_6$ and $P2Y_{11}$ receptors was also found. P2X1, P2X4 and P2X7 receptors were also present on the smooth muscle of human chorionic blood vessels [408].

Cytotrophoblast cells isolated from human term placenta were maintained in culture for 18 h (relatively undifferentiated mononuclear cells) or 66 h (differentiated multinucleate syncytiotrophoblast-like cells) [340]. P2X1 and P2X4, but not P2X3 or P2X5, receptors were expressed by all the cell types studied, while P2X2 receptors were found to be present in term placenta, and P2X7 receptors were found in both the first trimester and term. P2X7 receptors mediate production of PLD in human placental trophoblasts, implying involvement in the regulation of trophoblast function [93]. The varying P2X2 and P2X7 receptor expression may be correlated with gestational development and cytotrophoblast cell differentiation. ATP stimulates placental 11β-hydroxysteroid dehydrogenase type 2 activity, which is believed to play a key role in foetal development [446].

In late human pregnancy there is an increase in placental 5'-nucleotidase, and it was suggested that this may be associated with enhanced oestrogen synthesis and facilitation of uterine contractions during labour [59]. 5'-Nucleotidase was localised on the external surface of the microvillous plasma membrane of the syncytiotrophoblast, perhaps playing a role in regulation of the foeto–placental–maternal microcirculation in the human term placenta [262].

ADP-degrading properties of the placenta were described [175]. Later, ATP diphosphohydrolase (or apyrase) was identified in human term placenta [81,198,316]. Two isoforms (enzymes I and II) of placental ecto-ATP diphosphohydrolases were later identified by cDNA cloning [263]. Four ATPases, which bind to ATP at the external surface of the membrane of syncytiotrophoblast brush boarder membranes, were described [54]. Adenosine deaminase activity is differentially regulated in the maternal and foetal placental compartments. There are marked spatial and temporal changes in the profile of adenosine deaminase distribution in the antimesometrial decidua and basal zone of the chorioallentoic placenta and a rapid transition from maternal to foetal expression in midgestation [213]. Placental adenosine deaminase has been shown to be important for foetal development in mice [39]. Multiple regulatory protein binding motifs are necessary for adenosine deaminase gene expression in the placenta [367].

A study was undertaken to evaluate the relationship between utero–placental circulatory insufficiency and the foeto–placental release of adenosine in pregnancies complicated by pre-eclampsia [449]. It was concluded that foetal plasma adenosine increases before utero–placental insufficiency becomes severe enough to cause generalised foetal hypoxia. It was postulated that enhanced adenosine formation in the foetus, umbilical cord vessels and particularly the placenta may, at least in part, contribute to the control and maintenance of placental blood flow. P2X receptors have been identified on the smooth muscle of umbilical vessels [45].

Pre-eclampsia is characterised by elevated maternal blood pressure, proteinuria and altered foetal growth. It has been suggested that the pathophysiology of pre-eclampsia begins with shallow trophoblast invasion leading to placenta hypoxia. Hypoxia is a potent stimulus for the release of ATP, which is rapidly broken down by ectonucleotidases to adenosine. Women with pre-eclampsia and foetuses show increased circulating concentrations of adenosine. Adenosine A_{2A} receptors expression is elevated in placental biopsies, villous explants and placental microvillous membranes [423]. A_{2B} receptors on microvascular endothelial cells have also been implicated in pre-eclampsia [108]. Adenosine A_{2A} receptor stimulation decreases NO synthesis in placental microvascular endothelial cells from women with pre-eclampsia and intrauterine growth retardation [107]. Post-translational modifications of the P2X4 receptor in the human placenta occur in preeclampsia and the authors propose that there is increased release of ATP in pre-eclampsia in response to hypoxia and oxidative/nitrative stress to act on P2X4 receptors to influence placental cell homeostasis [343].

Evidence has been presented that suggests that placental adenosine mediates the placental disturbances elicited by alcohol, which may contribute to the pathogenesis of foetal alcohol syndrome [3].

NO released from endothelial cells following occupation of P2 receptors in response to ATP and ADP may play a major role in control of foetal vessel tone in human placenta and NOS activity is markedly reduced in pre-eclampsia. NO may also regulate the release of corticotrophin-releasing hormone from human placental syncytiotrophoblast cells. However, while it was considered that NO released from syncytiotrophoblasts prevented platelet aggregation in both maternal and foetal circulations, there is no evidence that platelet aggregating agents other than ADP can affect the production of NO [90].

The $P2Y_6$ receptor has been shown to be expressed in normal placenta and in gestational trophoblastic disease [381]. The authors showed that $P2Y_6$ mRNA production is highly characteristic of the epithelial-like cytotrophoblast and syncytiotrophoblast, whereas expression is absent in the mesenchymal-like intermediate trophoblast, suggesting that $P2Y_6$ receptors may play an important role in trophoblast development, differentiation and neoplasia.

Vagina

P2X5 and P2X7 receptors have been localised immunohistochemically in the stratified squamous epithelium of rat vagina, and it was suggested that they play a role in the physiological turnover of continuously regenerating cells [28,156]. Adenosine A_{2A} and P2 receptors are present in the wall of the rabbit vagina that mediate relaxation, but it was suggested that they are not involved in the NANC relaxant responses that remained after removal of NO transmission by the NOS inhibitor, L-NAME [459,460].

$P2Y_2$ receptor-selective agonists stimulate vaginal moisture in ovariectomised rabbits and $P2Y_2$ receptor mRNA is localised in stratified squamous epithelium of the vagina [276]. It was suggested that $P2Y_2$ receptor agonists produce a potential non-hormonal alternative for treating vaginal dryness in postmenopausal women. In the human vagina, similar to the ectocervix and epidermis, the P2X7 receptor plays an important role in the regulation of terminal differentiation and apoptosis [150].

Mammary glands

The epithelial cells lining the ducts of the human mammary gland are responsible for modifying sodium and potassium concentrations in milk by actively absorbing Na^+ from and secreting K^+ into the ductal fluid. ATP increased $[Ca^{2+}]_i$ leading to contraction of cultured mouse mammary myoepithelial cells [298]. These effects were unaffected by Ca^{2+} removal, suggesting that P2Y receptors were involved and the responses were blocked by suramin. In a later paper from this group, synergistic effects of ATP on oxytocin-

induced increases in $[Ca^{2+}]_i$ were demonstrated in these cells and it was suggested that purinoceptors may facilitate myoepithelial cell contraction in milk-ejection responses [299]. In another study, ATP and growth hormone were shown to cause prompt responses in H^+ efflux rate and increase in $[Ca^{2+}]_i$ in an opposite direction by treatment with lactogenic hormones [190]. Mechanically-induced calcium waves mediating purinergic coupling between myo- and secretary epithelial cells in mammary glands have been reported [140]. P2Y$_2$ receptors on the apical and basolateral membranes of 31EG4 mammary epithelia mediate apical Ca^{2+}-dependent Cl^- channels and cause fluid secretion that appears to be involved in the regulation of milk composition in vivo [43]. P2Y receptor regulation of sodium transport in human mammary epithelial cells has been described [229]. Adenosine, in concert with epidermal growth factor, stimulates mammary epithelial cell growth and DNA synthesis [454]. ATP and UTP stimulate Na^+ absorption and K^+ secretion via P2Y$_2$ receptors that increase $[Ca^{2+}]_i$ and activate Ca^{2+}-dependent K^+ channels [315]. They showed further that addition of the nucleotides to the luminal surface stimulated K^+ secretion, while their action on the basolateral surface led to increase in Na^+ absorption. Mastitis in cows is a problem to the dairy industry. A high level of ATP was found to be associated with mastitis in the udders of cows [302]. Ca^{2+}-ATPase was elevated in mammary tissue and milk fat globule membranes in cows with milk fever [326]. There is upregulation of P2 receptors in breast cancer (see [62]).

Conclusions

There is widespread functional expression of purinoceptors in reproductive organs: P2X1 and P2X2 receptors mediate contraction of smooth muscle in organs and blood vessels; P2X2 receptors mediate capacitation of sperm; P2X3 receptors mediate activation of sensory nerve fibres involved in reflex activities and pain; P2X4 receptors mediate luminal acidification in the epididymis and vas deferens, and probably also in the vagina and ectocervix; P2X5 receptors mediate differentiation and consequently have antiproliferative effects; P2Y$_1$ and P2Y$_2$ receptors mediate cell proliferation; P2Y$_2$ and P2X4 mediate regulation of epithelial transport in female reproductive tract epithelia; and P2X7 receptors mediate terminal differentiation and apoptotic cell death in epithelia.

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