British Journal of Pharmacology (2001) 132, 709–721

www.nature.com/bjp

The effects of P2Y receptor agonists and adenosine on prostaglandin production by the guinea-pig uterus

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1 This study has investigated the effects adenosine 5′-triphosphate (ATP), analogues of ATP, uridine 5′-triphosphate (UTP) and adenosine on prostaglandin output from the guinea-pig uterus superfused *in vitro*, and from guinea-pig endometrium and myometrium cultured for 24 h.

2 ATP, 2-methylthio ATP and adenosine increased the outputs of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and 6-keto-$PGF_{1\alpha}$ (reflecting PGI_2 production), and UTP increased the output of $PGF_{2\alpha}$ from the superfused guinea-pig uterus. These findings support the hypothesis that the contractile effects of ATP, 2-methylthio ATP, UTP and adenosine are mediated by prostaglandins.

3 Suramin (a P2 receptor antagonist) and 8-sulphophenyltheophylline (an A receptor antagonist) blocked the stimulatory actions of ATP and adenosine, respectively, on $PGF_{2\alpha}$ output, suggesting that ATP acts on P2 receptors (probably of the P2Y type) and adenosine acts on A receptors in the guinea-pig uterus to increase $PGF_{2\alpha}$ production.

4 ATP, 2-methylthio ATP, α,β-methylene ATP, β,γ-methylene ATP, UTP and adenosine increased the output of $PGF_{2\alpha}$ from guinea-pig endometrium and myometrium after 24 h of culture, with a greater stimulatory effect being exerted on the endometrium than on the myometrium. Little or no stimulatory effect was seen after 2 and 8 h of culture. In addition the effects of ATP, ATP analogues, UTP and adenosine on the outputs of PGE_2 and 6-keto-$PGF_{1\alpha}$ from cultured endometrium and myometrium were more variable, with both stimulation and inhibition being observed.

5 The stimulatory effects of ATP and adenosine on $PGF_{2\alpha}$ output from the endometrium and myometrium were associated with an increase in the prostaglandin synthesizing capacity of both tissues, due probably to an increase in the amount of prostaglandin H synthase present.

British Journal of Pharmacology (2001) **132,** 709–721

Keywords: Uterus; prostaglandins; ATP; uridine 5′-triphosphate; adenosine; suramin

Abbreviations: ATP, Adenosine 5′-triphosphate; NSAIDs, non-steroidal anti-inflammatory drugs; PG, prostaglandin; UTP, uridine 5′-triphosphate

Introduction

Adenosine 5′-triphosphate (ATP) and adenosine cause concentration-dependent contractions of the guinea-pig uterus (Moritoki *et al*., 1979). Treatment of the uterus with non-steroidal anti-inflammatory drugs (NSAIDs) inhibits the contractions produced by ATP and adenosine, without affecting contractions produced by acetylcholine and bradykinin. The inhibition of the response to ATP by NSAIDs is restored on the addition of arachidonic acid or prostaglandins. Moritoki *et al*. (1979) concluded that the contractile actions of ATP and adenosine are mediated by prostaglandins produced by the smooth muscle (i.e. the myometrium). These effects of ATP and adenosine on the guinea-pig uterus are mediated by receptors of the P_2 and A_1 types (Smith *et al*. 1988). On strips of guinea-pig myometrium (from which the endometrium has been removed), indomethacin fails to inhibit the contractions induced by adenosine, suggesting prostaglandin (PG) generation is not involved in the response (Bradley *et al*., 1993). Notwithstanding, the treatment of guinea-pig uterine strips with indomethacin abolishes the contractile responses produced by ATP analogues and uridine 5′-triphosphate (UTP), and inhibits the contractile response produced by ATP. Furthermore, removal of the endometrium from the uterine strips reduces the contractile responses to ATP, analogues of ATP, and UTP (Piper & Hollingsworth, 1996). All these studies suggest that the activation of P2Y receptors and, possibly A_1 receptors, in the guinea-pig uterus produces contractions of the myometrium by a process that involves prostaglandin generation largely by the endometrium. It is known that the endometrium is the major source of prostaglandin production in the guinea-pig uterus (Poyser, 1983a). Consequently the effects of ATP, analogues of ATP, UTP and adenosine on prostaglandin production by the guinea-pig uterus have been studied in order to investigate whether the contractile actions of these compounds on the guinea-pig uterus may be mediated by prostaglandins.

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Methods

Virgin, Dunkin-Hartley guinea-pigs (600–900 g) were examined daily and a vaginal smear was taken when the vagina was open. Day 1 of the cycle was taken as the day preceding the post-ovulatory influx of leukocytes when cornification was a maximum. All guinea-pigs had exhibited at least two cycles of normal length (about 16–17 days) before being killed (by stunning and incising the neck) on day 7 of the cycle. Each uterus was removed and separated into its two uterine horns. Each horn was 'opened' by cutting longitudinally along the anti-mesometrial side, and the horns were used in one of the following experiments.

Superfusion experiments

Each uterine horn was weighed, and was suspended in an organ bath with one end attached to an isotonic lever under a tension of 2 g. Each horn was superfused independently with Krebs solution (5 ml min^{-1}) at 37°C, as described by Poyser & Brydon (1983), and was superfused initially for a settling period of 60 min. In the first series of experiments, samples of superfusate were collected from both uterine horns every 10 min for the next 80 min (eight samples per uterine horn).

One of the uterine horns from each animal (five animals per treatment) was untreated (control). The other uterine horn (test) from each animal was treated during the collection of samples 4 and 5 (i.e. 30–50 min of sample collection) with ATP (100 μmol l^{-1}), 2-methylthio ATP (1 μmol l^{-1}), α,β-methylene ATP (100 μmol l^{-1}), β,γ-methylene ATP (100 μmol l^{-1}), UTP (100 μmol l^{-1}) or adenosine (100 μmol l^{-1}). The concentration chosen for each compound had previously been shown to produce maximal or near maximal contraction of the guinea-pig uterus (Moritoki *et al*., 1979; Piper & Hollingsworth, 1996). In the second series of experiments, the test uterine horn (five guinea-pigs per treatment) was treated with either suramin (100 μmol l^{-1}; a P2 receptor antagonist) or 8-sulphophenyltheophylline (140 μmol l^{-1}; an adenosine A receptor antagonist) during the collection of samples 4–7. In addition, the test uterine horn was treated with either ATP (100 μmol l^{-1}) or adenosine (100 μmol l^{-1}) during the collection of samples 6 and 7. The control uterine horn from each animal was untreated.

After collection, the pH of each sample of superfusate was lowered to 4.0 with M HCl and the prostaglandins were extracted by shaking twice with 50 ml ethyl acetate. The two ethyl acetate fractions were combined and evaporated to dryness on a rotary evaporator at 50°C. The recoveries of $PGF_{2\alpha}$ and PGE_2 by this method are >90%, and the recovery of 6-keto-$PGF_{1\alpha}$ is >80% (Poyser & Scott, 1980; Swan & Poyser, 1983). Each dried extract was re-dissolved in 10 ml ethyl acetate and stored at −20°C before the prostaglandin content was measured by radioimmunoassay (RIA).

Tissue culture experiments

For each uterine horn, the endometrium was separated from the myometrium by cutting away 1–2 mm^3 pieces of endometrium with a pair of fine scissors. The myometrium was also cut into similar-sized pieces. This technique produces >85% separation of the two tissues (Leaver & Poyser, 1981). Pieces of endometrium or myometrium were placed on a raised platform in a Petri dish (10–40 mg tissue per dish) that contained 4 ml Medium 199 (plus Earle's salts) supplemented with amphotericin B (1.5 μg ml^{-1}), L-glutamine (1.6 mmol l^{-1}) and kanamycin (30 μg ml^{-1}). The endometrium and myometrium were treated with ATP (10, 50 and 100 μmol l^{-1}), 2-methylthio ATP (1, 5, 10 and 25 μmol l^{-1}), α,β-methylene ATP (10, 50 and 100 μmol l^{-1}), β,γ-methylene ATP (10, 50 and 100 μmol l^{-1}), UTP (10, 50 and 100 μmol l^{-1}) or adenosine (10, 50 and 100 μmol l^{-1}). The concentrations of each compound used reflected the range of the spasmogenic responses (Piper & Hollingsworth, 1996). Six 'treated' Petri dishes plus two Petri dishes containing tissue but no treatments (controls) were placed in a modified Kilner jar, and the tissues were cultured for 24 h as described by Ning *et al*. (1983) and Ning & Poyser (1984). The culture medium was collected after 2, 8 and 24 h and was replaced with culture medium containing the same treatment. Each sample of culture medium was stored at −20°C, and the amounts of prostaglandins present were measured without extraction by RIA. Following culture, the tissue in each dish was blotted dry and weighed.

The amounts of prostaglandins produced by homogenates of the endometrium and myometrium after 24 h culture in the absence and presence of ATP (100 μmol l^{-1}) or adenosine (100 μmol l^{-1}) were also measured. Each tissue type was homogenized in 10 ml Krebs solution using a Fisons glass homogenizer and incubated for 60 min at 37°C for 60 min. The pH of each incubate was then lowered to pH 4 with M HCl and the prostaglandins extracted twice with 20 ml ethyl acetate. The ethyl acetate fractions were combined and evaporated to dryness as described before. Each residue was re-dissolved in 5 ml ethyl acetate, and was stored at −20°C before the prostaglandin content was measured by RIA.

Assays

The amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ present in each sample were measured by RIA using antibodies raised in this laboratory; the cross-reactivities have been reported elsewhere (Poyser, 1987). When tissue culture samples were assayed, an equivalent volume of culture medium was incorporated into the standard prostaglandin solutions used in the assay. The intra-assay and inter-assay coefficients of variation were <12% for all three assays. The detection limit was 10–30 pg prostaglandin per assay tube. Since prostaglandins are not stored in the guinea-pig uterus (Poyser, 1972), the amounts of prostaglandins released into the superfusing solution or tissue culture medium and formed by homogenates during incubation reflect fresh prostaglandin synthesis, particularly as indomethacin exerts an inhibitory effect (Poyser, 1972, 1985; Riley & Poyser, 1990).

Sources of material

Suramin, ATP, 2-methylthio ATP, α,β-methylene ATP, β,γ-methylene ATP, UTP and adenosine were purchased from Sigma–Aldrich Co. Ltd, Poole, U.K.; 8-sulphophenyltheophylline was purchased from RBI, St Albans, U.K.; Medium 199 (plus Earle's salts), amphotericin B, L-glutamine and kanamycin were purchased from Flow Laboratories, Irvine, U.K.

Statistical tests

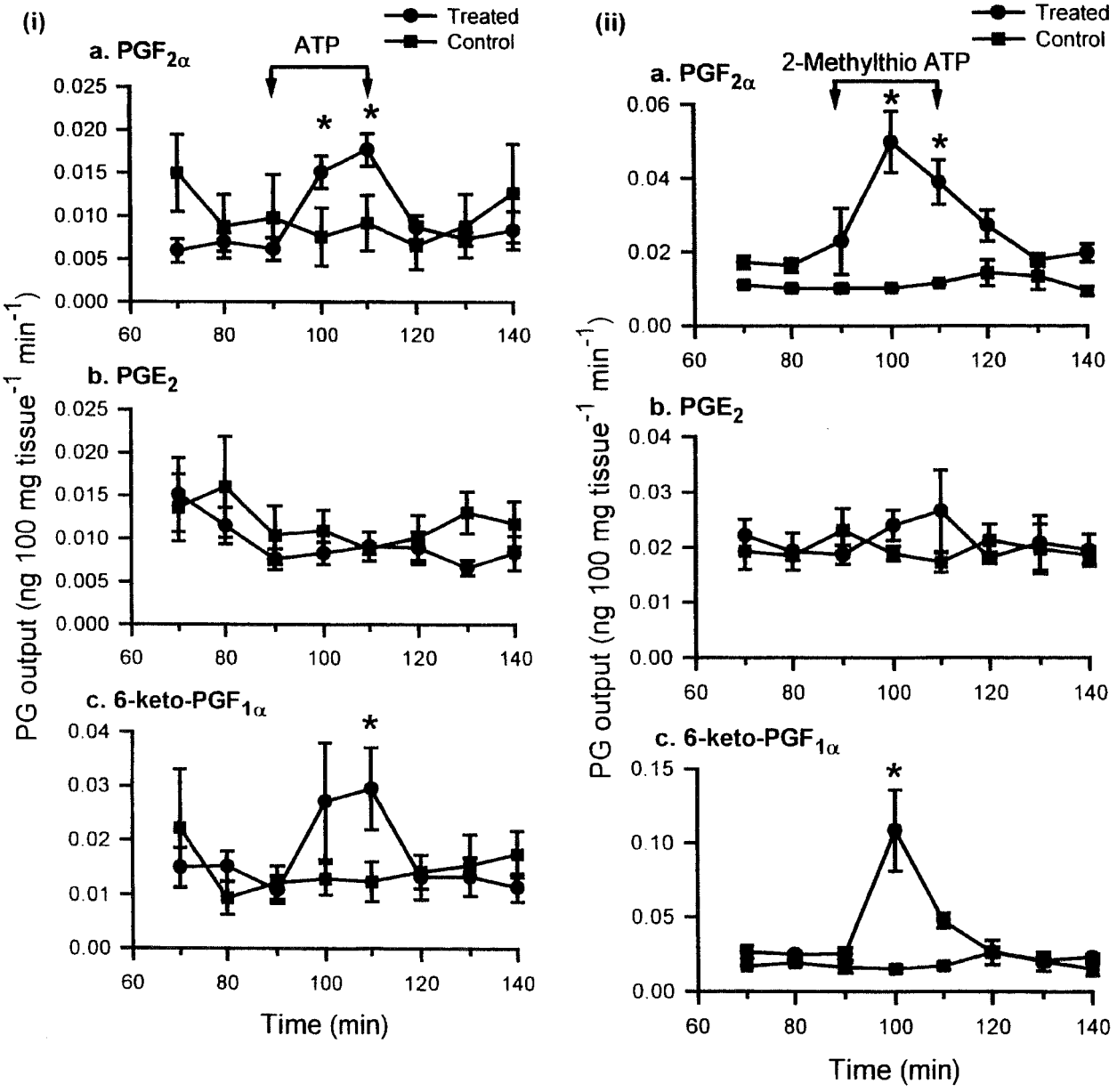
In the superfusion experiments, changes in the output of prostaglandins with time were analysed by Duncan's multiple range test. In the tissue culture experiments, the two control samples per Kilner jar were averaged. The results were analysed by one-way analysis of variance (ANOVA) and by the paired *t*-test.

Results

Superfusion experiments

ATP (100 μmol l^{-1}), 2-methylthio ATP (1 μmol l^{-1}), UTP (100 μmol l^{-1}) and adenosine (100 μmol l^{-1}) significantly ($P<0.05$) increased the output of $PGF_{2\alpha}$ from the day 7 guinea-pig uterus superfused *in vitro* (Figure 1). The outputs of $PGF_{2\alpha}$ during treatment with α,β-methylene ATP (100 μmol l^{-1}) and β,γ-methylene ATP (100 μmol l^{-1}) tended to rise, but the outputs were not significantly changed (Figure 1). The output of PGE_2 from the guinea-pig uterus was unaffected by ATP, 2-methylthio ATP, α,β-methylene ATP, β,γ-methylene ATP, UTP and adenosine (Figure 1). The output of 6-keto-$PGF_{1\alpha}$ from the guinea-pig uterus was increased significantly ($P<0.05$) by ATP, 2-methylthio ATP and adenosine, but not by α,β-methylene ATP, β,γ-methylene ATP and UTP (Figure 1).

Suramin and 8-sulphophenyltheophylline alone had no effect on the basal outputs of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ from the guinea-pig uterus (Figure 2). However, suramin inhibited the increase in $PGF_{2\alpha}$ output from the guinea-pig uterus induced by ATP (Figure 2.i) but not by adenosine (Figure 2.iii). Conversely, 8-sulphophenyltheophyl-

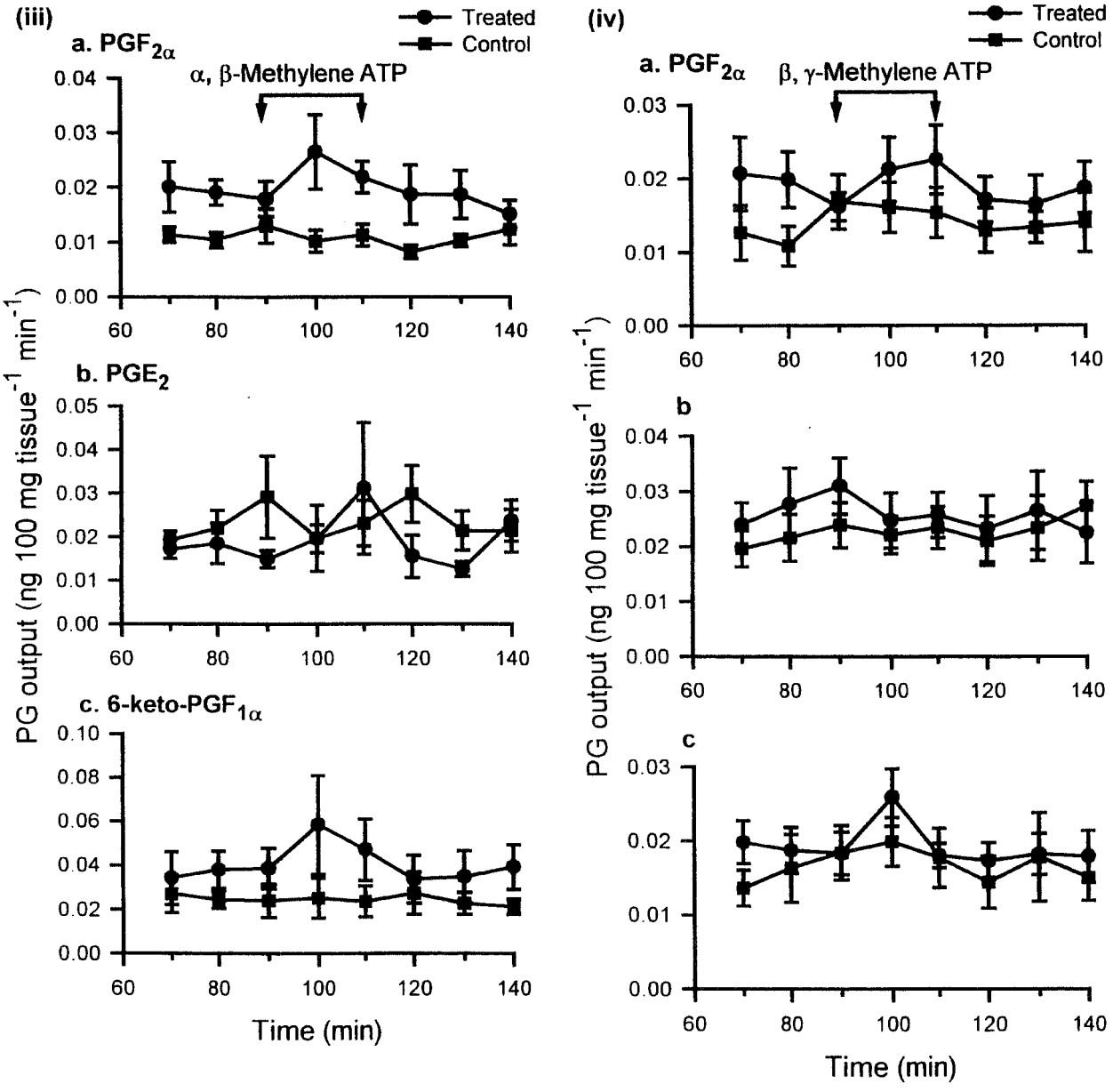


line inhibited the increase in $PGF_{2\alpha}$ output induced by adenosine (Figure 2.iv) but not by ATP (Figure 2.ii). Neither suramin nor 8-sulphophenyltheophylline prevented the significant increases in the output of 6-keto-$PGF_{1\alpha}$ induced by ATP and adenosine (Figure 2).

Tissue culture experiments

Endometrium in culture The major prostaglandin released from guinea-pig endometrium in culture was 6-keto-$PGF_{1\alpha}$, together with lesser quantities of $PGF_{2\alpha}$ and PGE_2. The outputs of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ significantly ($P<0.05$) decreased during the 24 h culture period (Figure 3). The output of $PGF_{2\alpha}$ was significantly ($P<0.05$) increased after 24 h of culture by ATP (10–100 μmol l^{-1}), 2-methylthio ATP (1–25 μmol l^{-1}), α,β-methylene ATP (10–100 μmol l^{-1}), β,γ-methylene ATP (10–100 μmol l^{-1}), UTP (10–100 μmol l^{-1}) and adenosine (10–100 μmol l^{-1}) in a non-concentration dependent (Figure 3). The output of $PGF_{2\alpha}$ was significantly ($P<0.05$) increased after 8 h of culture by α,β-methylene ATP (50 and 100 μmol l^{-1}) and β,γ-methylene ATP (50 and 100 μmol l^{-1}), but not by ATP, 2-methylthio ATP, UTP and adenosine. None of the compounds tested increased $PGF_{2\alpha}$ output after 2 h of culture (Figure 3).

The output of PGE_2 was significantly ($P<0.05$) increased after 24 h of culture by 2-methylthio ATP (1 μmol l^{-1}), α,β-methylene ATP (10–100 μmol l^{-1}), β,γ-methylene ATP (10–100 μmol l^{-1}) and adenosine (100 μmol l^{-1}), but not by ATP (10–100 μmol l^{-1}), 2-methylthio ATP (5–25 μmol l^{-1}), UTP (10–100 μmol l^{-1}) and adenosine (50 and 100 μmol l^{-1}; Figure 3). None of the compounds increased PGE_2 output



after 2 and 8 h of culture (Figure 3). In fact, PGE_2 output was significantly ($P<0.05$) decreased by 2-methylthio ATP (5 μmol l^{-1}) and UTP (100 μmol l^{-1}) after 8 h of culture, and by β,γ-methylene ATP (50 μmol l^{-1}), UTP (10 μmol l^{-1}) and adenosine (50 and 100 μmol l^{-1}) after 2 h of culture (Figure 3)

The output of 6-keto-$PGF_{1\alpha}$ was significantly ($P<0.05$) decreased by ATP (10–100 μmol l^{-1}) and 2-methylthio ATP (5 and 10 μmol l^{-1}) after 8 and 24 h of culture, and by α,β-methylene ATP (50 and 100 μmol l^{-1}) after 8 h of culture (Figures 3.i, ii). Conversely, the output of 6-keto-$PGF_{1\alpha}$ was significantly ($P<0.05$) increased by β,γ-methylene ATP (50 μmol l^{-1}) and adenosine (10 and 50 μmol l^{-1}) after 24 h of culture (Figures 3.iii, iv). None of the compounds affected 6-keto-$PGF_{1\alpha}$ output during the first 2 h of culture (Figure 3).

Myometrium in culture

The major prostaglandin released from guinea-pig myometrium in culture was 6-keto-$PGF_{1\alpha}$, together with lesser quantities of $PGF_{2\alpha}$ and PGE_2. The outputs of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ significantly ($P<0.05$) decreased during the 24 h culture period (Figure 4). The output of $PGF_{2\alpha}$ was significantly ($P<0.05$) increased after 24 h of culture by ATP (10 μmol l^{-1}), 2-methylthio ATP (1 and 25 μmol l^{-1}), α,β-methylene ATP (10–100 μmol l^{-1}), β,γ-methylene ATP (10–100 μmol l^{-1}), UTP (10–100 μmol l^{-1}) and adenosine

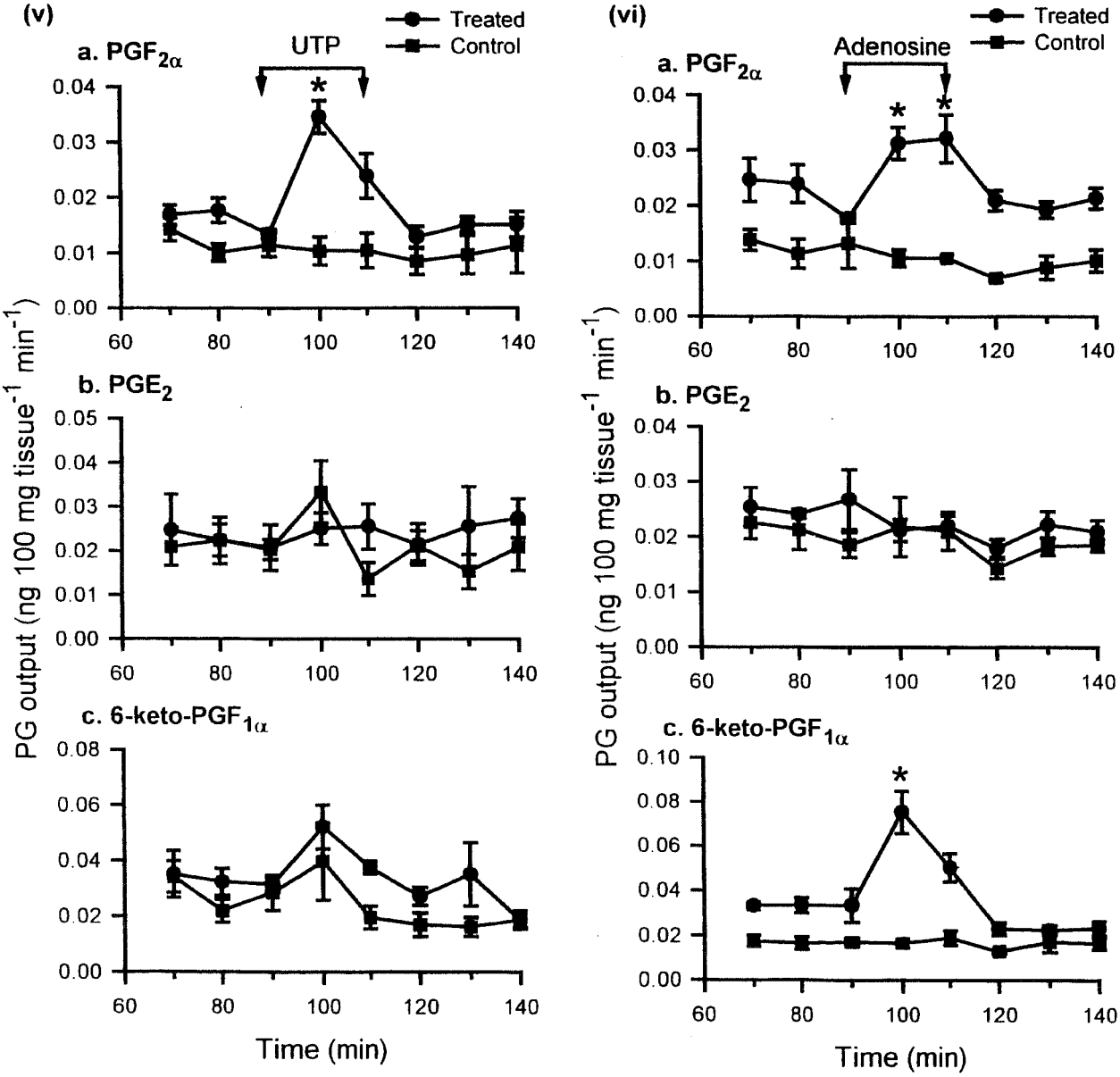


Figure 1 Mean (±s.e. mean, $n=5$) outputs of (a) prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), (b) PGE_2 and (c) 6-keto-$PGF_{1\alpha}$ from the guinea-pig uterus superfused *in vitro* in the absence (Control) and presence (Treated) of (i) ATP, (ii) 2-methylthio ATP, (iii) α,β-methylene ATP, (iv) β,γ-methylene ATP, (v) UTP, and (vi) adenosine. *Significantly ($P<0.05$) higher than value immediately preceding treatment.

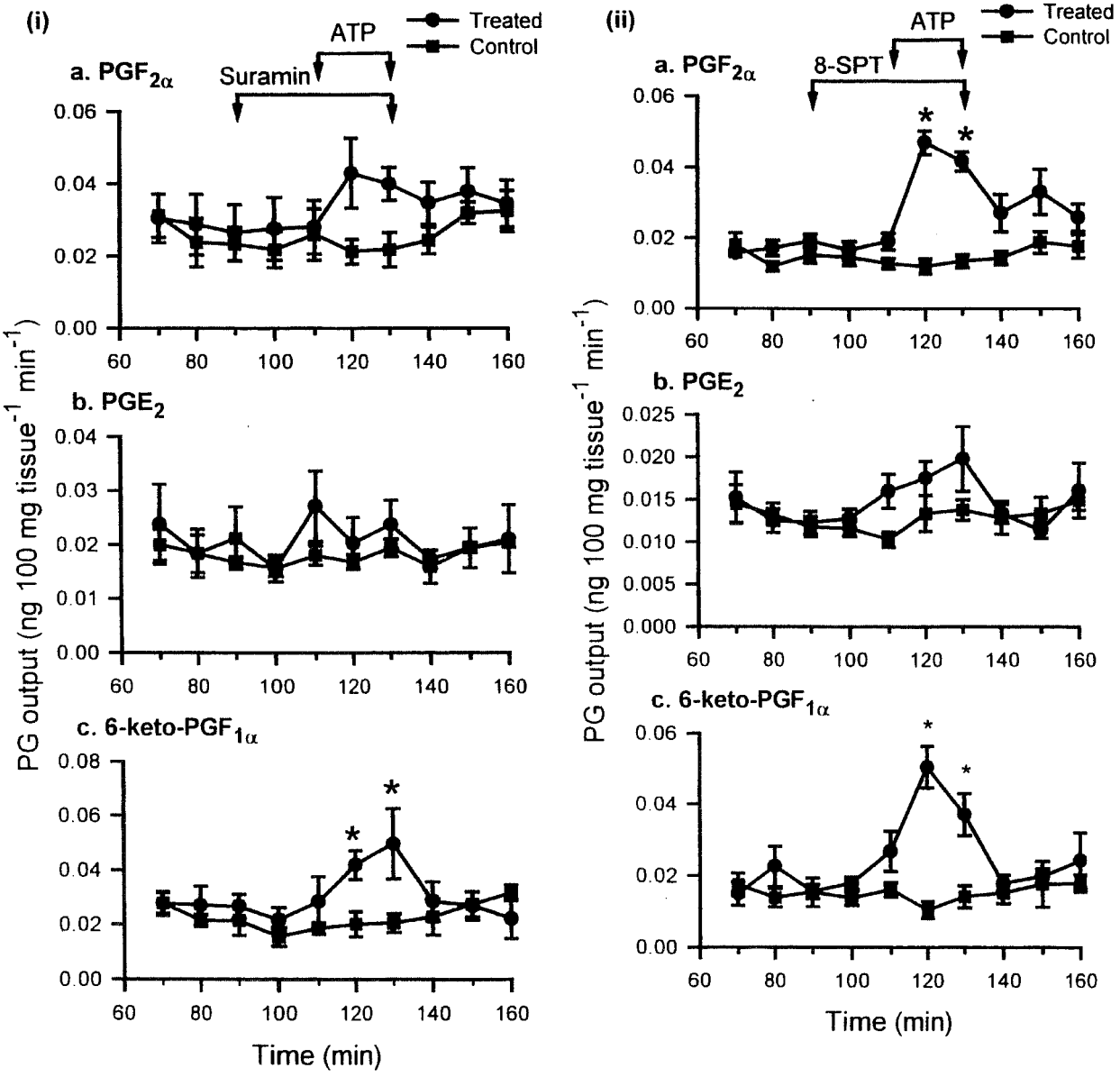
(50 μmol l^{-1}; Figure 4). None of the compounds increased $PGF_{2\alpha}$ output during the first 2 h of culture, while only α,β-methylene ATP (50 μmol l^{-1}) and β,γ-methylene ATP (10 and 50 μmol l^{-1}) significantly ($P<0.05$) increased $PGF_{2\alpha}$ output after 8 h of culture (Figure 4).

The output of PGE_2 was significantly ($P<0.05$) decreased by β,γ-methylene ATP (10 and 100 μmol l^{-1}) after 2 h of culture (Figure 4.iii), by ATP (100 μmol l^{-1}) and 2-methylthio ATP (5 and 10 μmol l^{-1}) after 8 h of culture, and by ATP (10 and 100 μmol l^{-1}) and 2-methylthio ATP (5 and 10 μmol l^{-1}) after 24 h of culture (Figure 4.i). The output of PGE_2 was significantly ($P<0.05$) increased by β,γ-methylene ATP (10 to 100 μmol l^{-1}) after 24 h of culture (Figure 4.iii). PGE_2 output was not affected by α,β-methylene ATP, UTP and adenosine (Figures 4.ii, iv).

The output of 6-keto-$PGF_{1\alpha}$ was significantly ($P<0.05$) decreased by ATP (10–100 μmol l^{-1}) and 2-methylthio ATP (5–25 μmol l^{-1}) after 8 and 24 h of culture (Figure 4.i), by α,β-methylene ATP (10 and 50 μmol l^{-1}) after 24 h (Figure 4.ii), and by adenosine (10 μmol l^{-1}) after 2 and 8 h of culture (Figure 4.iv). The output of 6-keto-$PGF_{1\alpha}$ was significantly ($P<0.05$) increased by α,β-methylene ATP (50 μmol l^{-1}) during the first 2 h of culture (Figure 4.ii). The output of 6-keto-$PGF_{1\alpha}$ was not affected by β,γ-methylene ATP and UTP (Figures 4.iii, iv).

Incubation of homogenates of cultured endometrium and myometrium

PGE_2 was the major prostaglandin synthesized during the incubation of homogenates of guinea-pig endometrium previously cultured for 24 h, together with lesser quantities of $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$. The treatment of the endometrium during culture with ATP (100 μmol l^{-1}) or adenosine (100 μmol l^{-1}) significantly ($P<0.05$) increased the amounts of $PGF_{2\alpha}$ produced, without significantly affecting PGE_2 and 6-



keto-$PGF_{1\alpha}$ production (Figure 5). $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ were produced in similar quantities by homogenates of cultured myometrium during incubation. The treatment of the myometrium during culture with ATP (100 μmol l^{-1}) or adenosine (100 μmol l^{-1}) significantly ($P<0.05$) increased the amounts of $PGF_{2\alpha}$ and PGE_2 produced, without significantly affecting 6-keto-$PGF_{1\alpha}$ production (Figure 5).

Discussion

The outputs of $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ (which reflects PGI_2 output), but not of PGE_2, from the day 7 guinea-pig uterus superfused *in vitro* were increased 2–3 fold by treatment with ATP, 2-methylthio ATP and adenosine. The output of $PGF_{2\alpha}$, but not of 6-keto-$PGF_{1\alpha}$ or PGE_2, was similarly increased by UTP. $PGF_{2\alpha}$ contracts the guinea-pig uterus and potentiates the contractile effect of ATP on guinea-pig myometrium (Dozi-Vassilides *et al*., 1976; Whalley & White, 1980a, b; Weichman & Tucker, 1982; Coleman & Parkington, 1988). PGI_2 contracts the rat uterus and potentiates the action of other spasmogens on this tissue (Vane & Williams, 1973; Williams *et al*., 1979; Phillips & Poyser, 1981). Consequently, the present findings are consistent with the hypothesis that the contractile actions of ATP, ATP, 2-methylthio ATP, adenosine and UTP on the guinea-pig uterus are mediated by prostaglandins, particularly $PGF_{2\alpha}$ and possibly also PGI_2. However, inconsistent with the hypothesis is the finding that α,β-methylene ATP and β,γ-methylene ATP failed to cause statistically significant

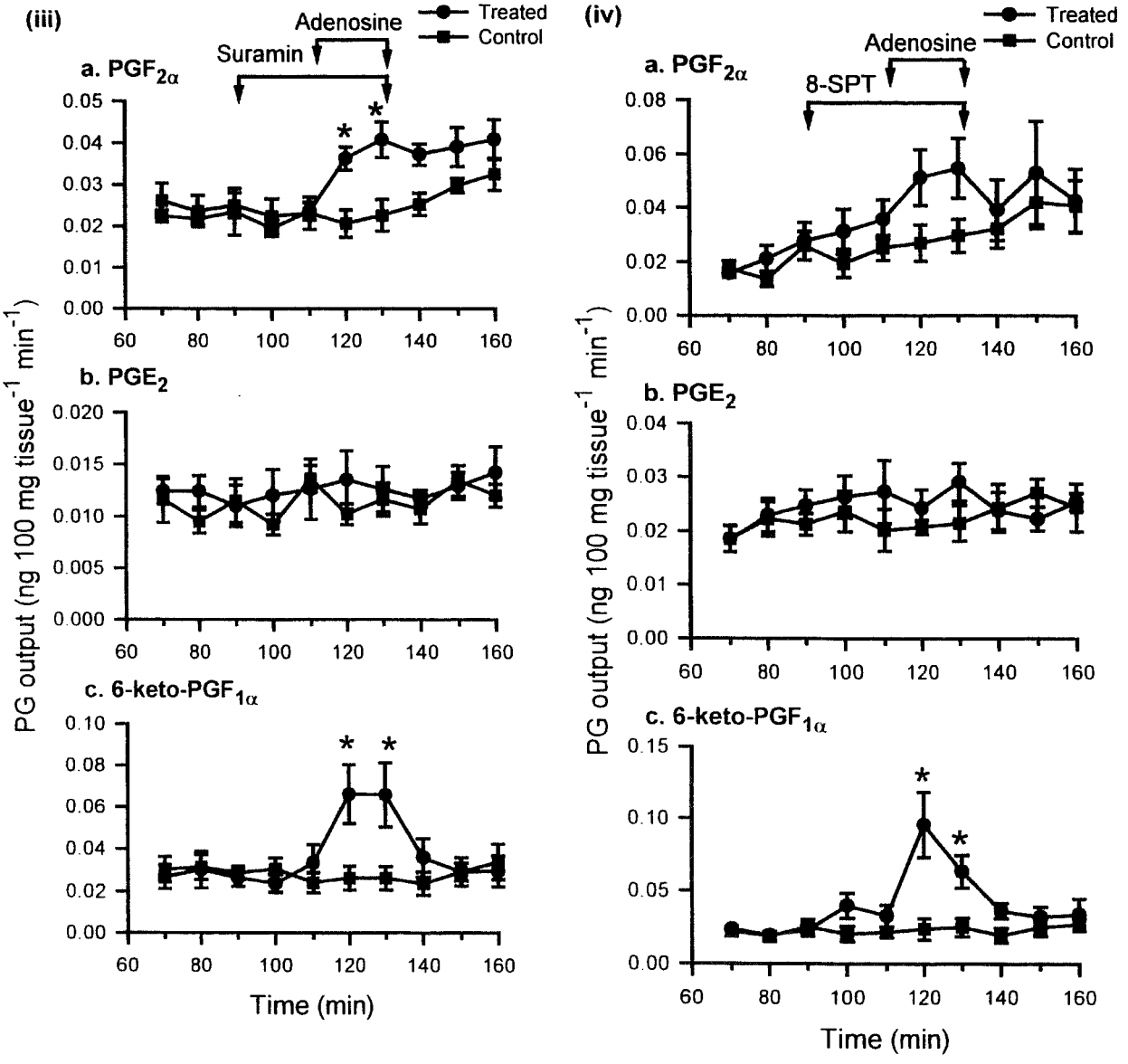
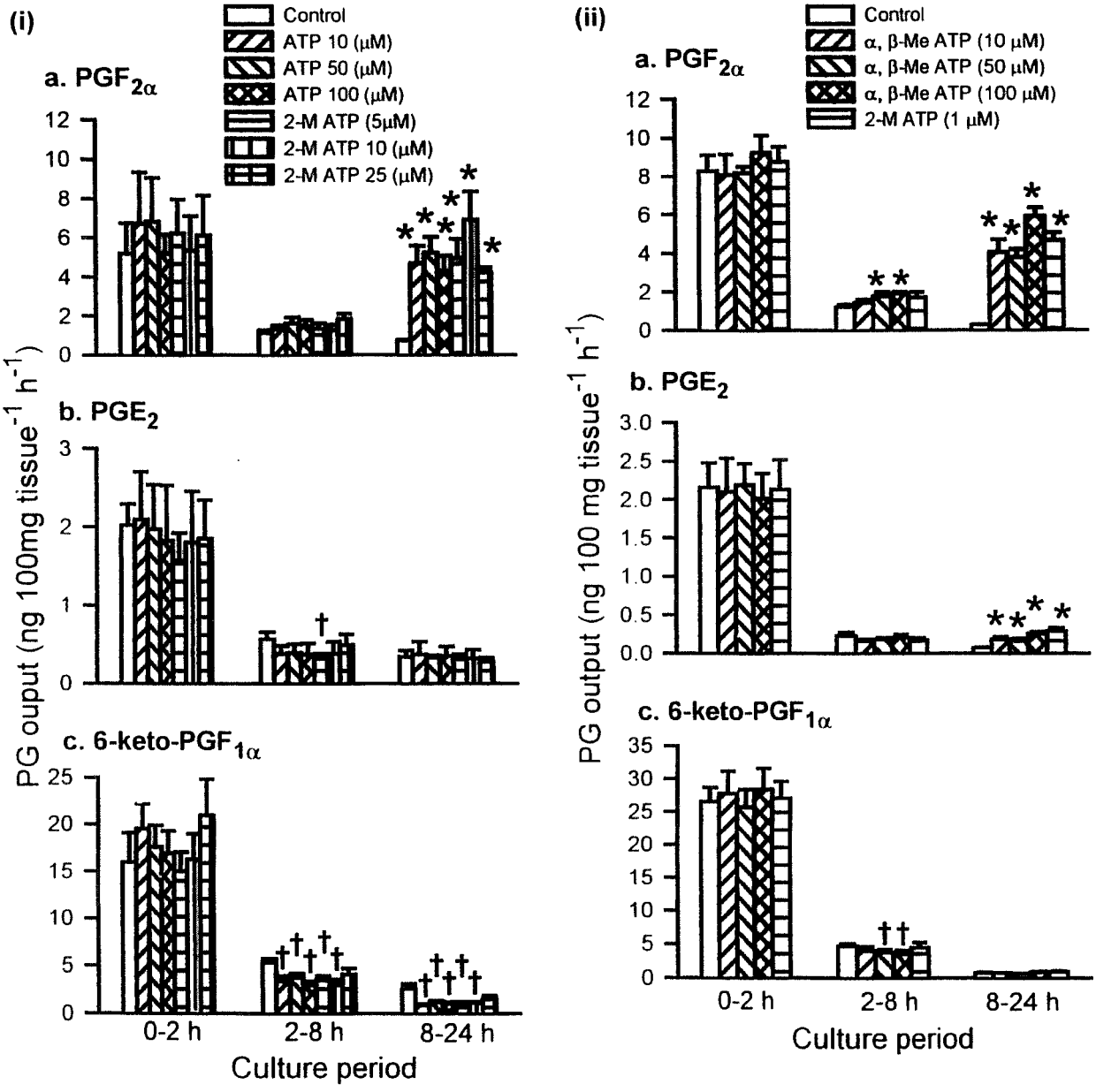


Figure 2 Mean (±s.e. mean, $n=5$) outputs of (a) prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), (b) PGE_2 and (c) 6-keto-$PGF_{1\alpha}$ from the guinea-pig uterus superfused *in vitro* in the absence (Contol) and presence (Treated) of (i) suramin plus ATP, (ii) 8-sulphophenyltheophylline (8-SPT) plus ATP, (iii) suramin plus adenosine, and (iv) 8-SPT plus adenosine. *Significantly ($P<0.05$) higher than value immediately preceding ATP or adenosine treatment.

increases in prostaglandin output from the superfused guinea-pig uterus, although the trend for both compounds was to increase the outputs of $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$. The release of arachidonic acid from phospholipids by the action of phospholipase A_2 (PLA_2) is the rate-limiting step in prostaglandin synthesis by the day 7 guinea-pig uterus superfused *in vitro* (Poyser, 1985, 1987). Therefore ATP, 2-methylthio ATP, UTP and adenosine probably increase uterine prostaglandin production by activating PLA_2, with the arachidonic acid released being directed into the $PGF_{2\alpha}$- and PGI_2- forming pathways.

Both ATP and adenosine increased the outputs of $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$, suggesting that P2 and A receptors are present in the guinea-pig uterus. However, it is possible that ATP is rapidly broken down to adenosine by ectonucleotidases and produces its effect by acting on A receptors and not on P2 receptors. Consequently the effects of suramin (a P2 receptor antagonist) and 8-sulphophenyltheophylline (an A receptor antagonist) on the increases in prostaglandin outputs produced by ATP and adenosine were examined. Suramin and 8-sulphophenyltheophylline treatment alone had no effect on prostaglandin output from the superfused uterus. However suramin, but not 8-sulphophenyltheophylline, prevented the increase in $PGF_{2\alpha}$ output induced by ATP, while 8-sulphophenyltheophylline, but not suramin, prevented the increase in $PGF_{2\alpha}$ output induced by adenosine. These findings suggest that ATP and adenosine stimulate increased $PGF_{2\alpha}$ production by the guinea-pig uterus by acting on P2 receptors (probably of the P2Y type; Piper & Hollingsworth, 1996) and A receptors, respectively. Surprisingly, neither antagonist prevented the increases in 6-keto-$PGF_{1\alpha}$ output produced by ATP and adenosine.



ATP, 2-methylthio ATP, α,β-methylene ATP, β,γ-methylene ATP, UTP and adenosine increased the output of $PGF_{2\alpha}$ from day 7 guinea-pig endometrium and myometrium after 24 h of culture. The average increase in $PGF_{2\alpha}$ output was 15.5 fold (range 5.9–30.5 fold) from the endometrium and 3.7 fold (range 1.4–7.7 fold) from the myometrium. There is no detectable output of the main metabolite of $PGF_{2\alpha}$ (13, 14-dihydro-15-keto-$PGF_{2\alpha}$) into the culture medium from the untreated tissues during 24 h of culture (Aitken & Poyser, unpublished observation). Therefore changes in prostaglandin output from the uterine tissues during culture reflect changes in prostaglandin synthesis. Consequently ATP, analogues of ATP, UTP and adenosine have a greater stimulatory effect on $PGF_{2\alpha}$ synthesis by the endometrium than by the myometrium. This is consistent with the findings that the endometrium is the probable source of the prostaglandins that mediate the contractile effects of these compounds on the guinea-pig uterus (Piper & Hollingsworth, 1996). The stimulatory effects of ATP, ATP analogues, UTP and adenosine on the outputs of PGE_2 and 6-keto-$PGF_{1\alpha}$ from cultured endometrium and myometrium were less marked and more variable than the effects on $PGF_{2\alpha}$ output, and in

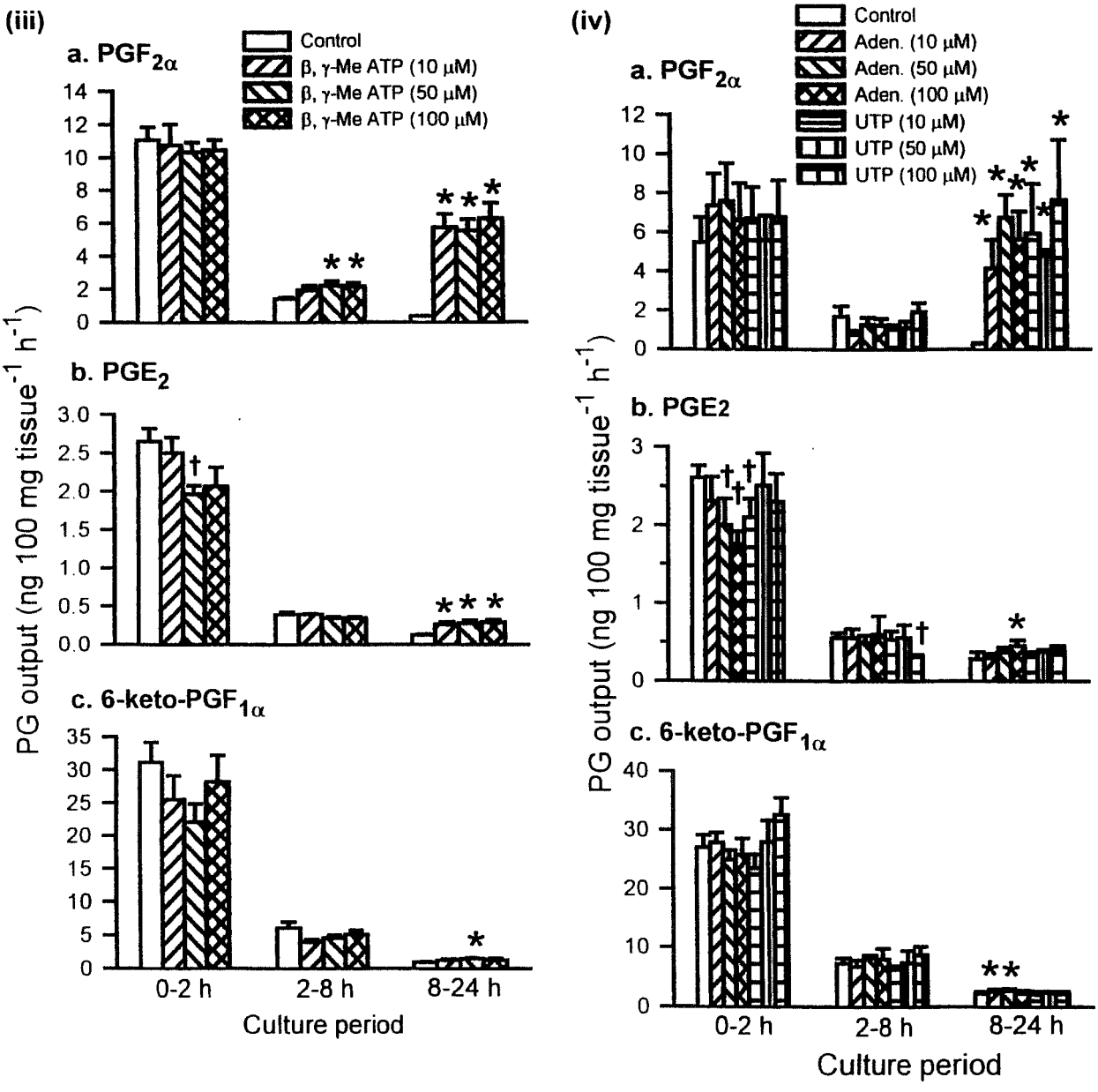
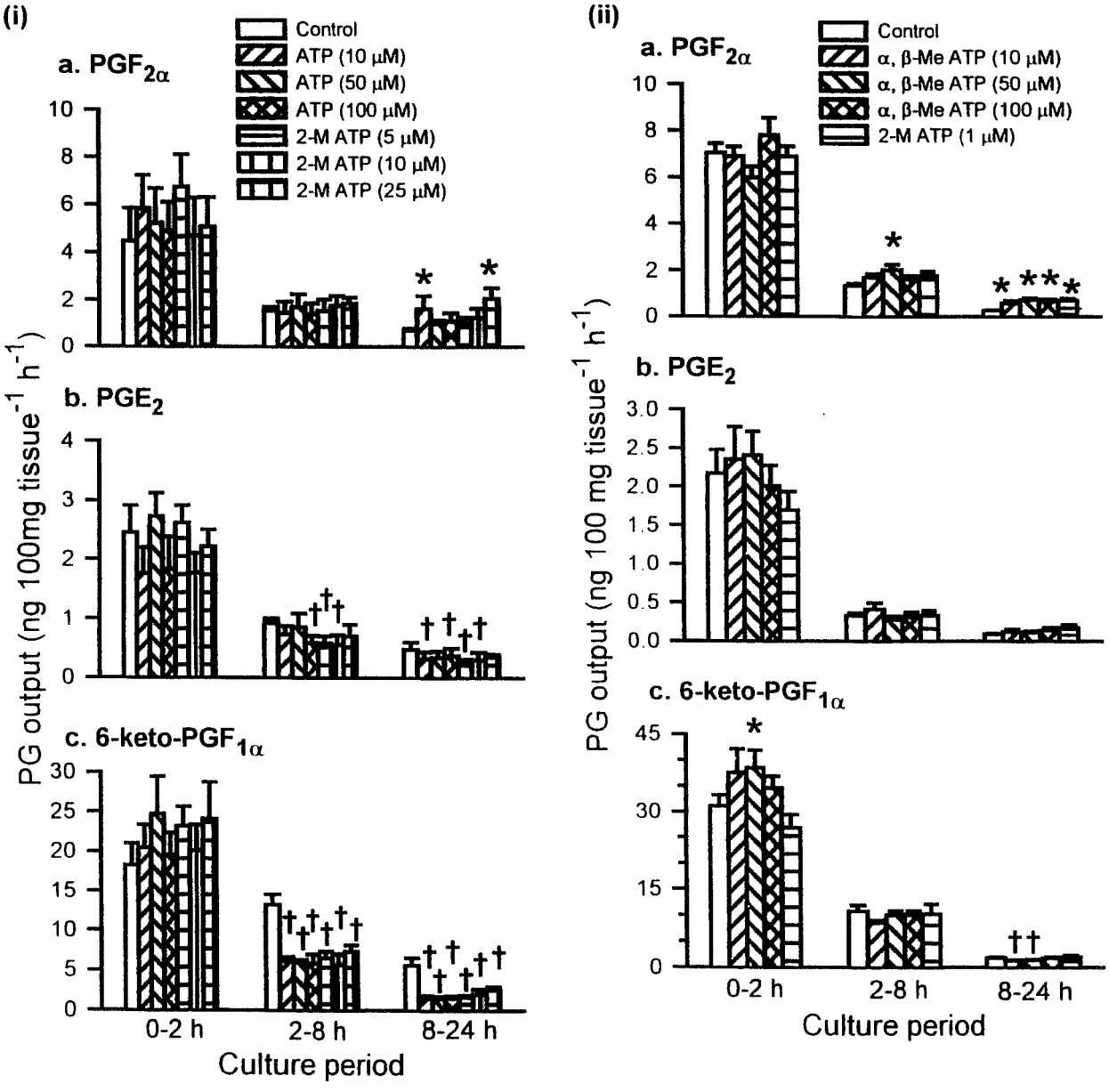


Figure 3 Mean (±s.e. mean, $n=5$) outputs of (a) prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), (b) PGE_2 and (c) 6-keto-$PGF_{1\alpha}$ from the guinea-pig endometrium cultured for 24 h in the absence (Control) and presence of (i) 10, 50 and 100 μmol l^{-1} ATP or 5, 10 and 25 μmol l^{-1} 2-methylthio ATP (2-M ATP), (ii) 10, 50 and 100 μmol l^{-1} α,β-methylene ATP (α,β-Me ATP) or 1 μmol l^{-1} 2-methylthio ATP (2-M ATP), (iii) 10, 50 and 100 μmol l^{-1} β,γ-methylene ATP (β,γ-Me ATP), and (iv) 10, 50 and 100 μmol l^{-1} adenosine (Aden.) or 10, 50 and 100 μmol l^{-1} UTP. *Significantly ($P<0.05$) higher than the corresponding control value in the same culture period. † Significantly ($P<0.05$) lower than the corresponding control value in the same culture period.

some instances inhibition of prostaglandin output was observed.

ATP, analogues of ATP, UTP and adenosine had no stimulatory effects on $PGF_{2\alpha}$ output from guinea-pig endometrium and myometrium after 2 h of culture, and had only weak stimulatory effect after 8 h of culture. It is probable that any acute stimulation of prostaglandin output similar to that observed in the superfusion experiments is masked by the more prolonged and continuous basal output occurring during the tissue culture experiments. Therefore, although it is likely that increased mobilization of arachidonic acid is the reason for increased prostaglandin synthesis by the superfused uterus, increased prostaglandin synthesis (particularly of $PGF_{2\alpha}$) by the uterine tissues after 24 h is partly due to a different mechanism. Since the concentration of free arachidonic acid is not rate-limiting for prostaglandin synthesis by homogenates of guinea-pig uterine tissues due to a large release of arachidonic acid from the disturbed cells (Mitchell *et al*., 1977), the amounts of prostaglandins produced by these tissues reflect the amount of prostaglandin H synthase present. The amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ synthesized by the homogenates of the endometrium following 24 h of culture were increased 3.6 fold and 4.1 fold when ATP and adenosine, respectively, were present in the culture medium. Likewise the amounts of the three prostaglandins synthesized by homogenates of cultured myometrium were increased 3.9 fold and 3.4 fold by ATP and adenosine respectively. These findings indicate that ATP and adenosine stimulate the synthesis of PGHS in both the endometrium and myometrium, with most of the PGH_2 formed being converted into $PGF_{2\alpha}$ by the endometrial homogenates, and into $PGF_{2\alpha}$ and PGE_2 by the myometrial



homogenates. Although the $PGF_{2\alpha}$ synthesizing capacities of these broken cell preparations of the endometrium and myometrium following 24 h of culture with ATP or adenosine are similar, the amounts of $PGF_{2\alpha}$ synthesized by and released from the endometrium are much greater than from the myometrium during the 8–24 h period of culture in the presence of ATP or adenosine. Therefore, in the intact tissues, it is likely that the supply of arachidonic acid is still rate-limiting for $PGF_{2\alpha}$ synthesis, and that ATP and adenosine stimulate a greater release from the endometrium than from the myometrium during the last culture period. The increase in PGHS concentration in the tissue means that more of the arachidonic acid released is converted into the $PGF_{2\alpha}$. It is well-documented that the guinea-pig endometrium when stimulated preferentially synthesises $PGF_{2\alpha}$ (Poyser, 1983b, 1995). It is likely that ATP and adenosine stimulate the production of PGHS-2 in the endometrium and myometrium as this is the main form of PGHS in the guinea-pig uterus (Naderali & Poyser, 1996).

Overall, this study has shown that ATP, 2-methylthio ATP, UTP and adenosine cause an acute stimulation of $PGF_{2\alpha}$ and PGI_2 production by the guinea-pig uterus superfused *in vitro*. It is possible, therefore, that prostaglandins mediate the contractile actions of these compounds on the guinea-pig

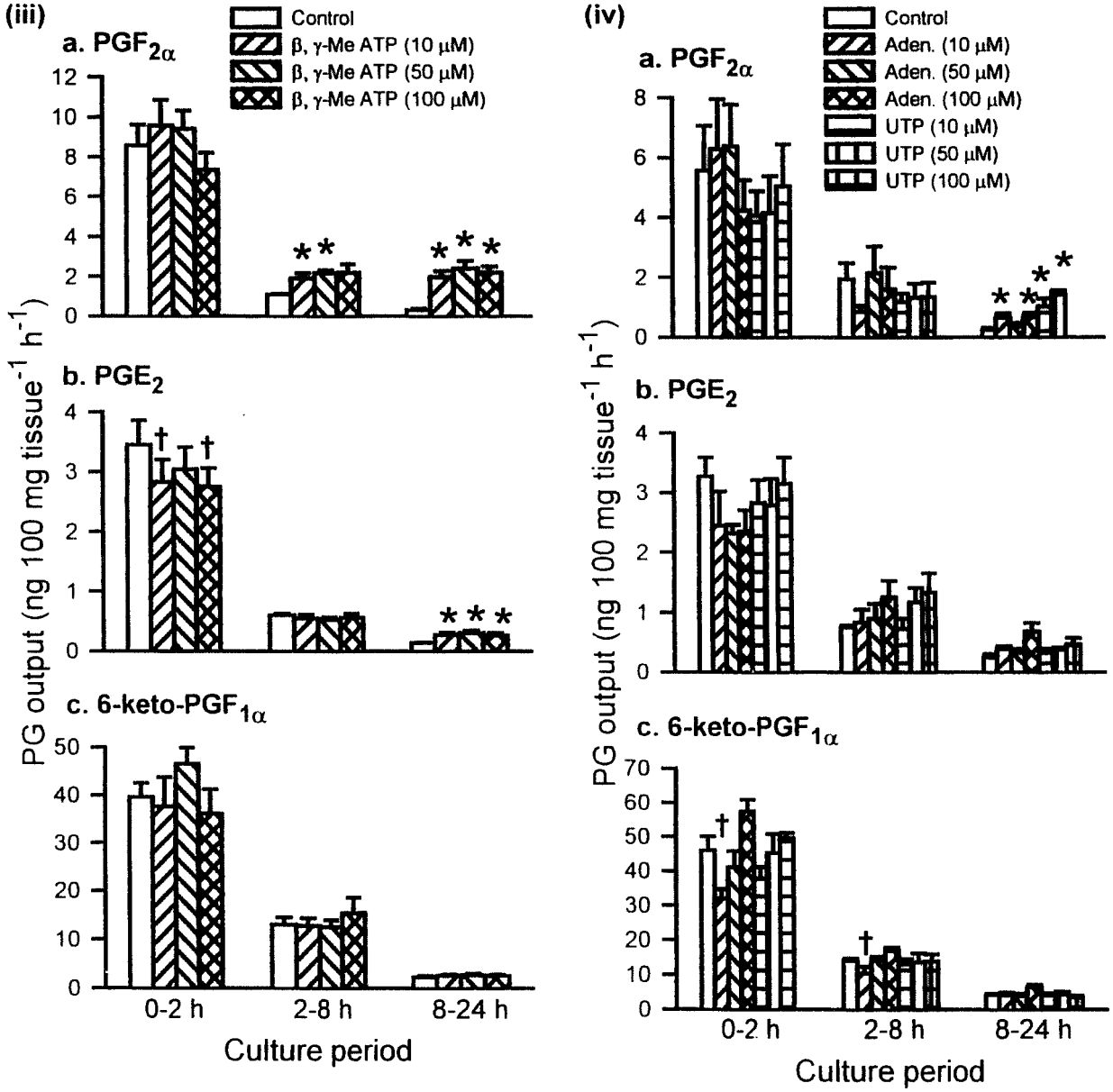


Figure 4 Mean (±s.e. mean, $n=5$) outputs of (a) prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), (b) PGE_2 and (c) 6-keto-$PGF_{1\alpha}$ from the guinea-pig myometrium cultured for 24 h in the absence (Control) and presence of (i) 10, 50 and 100 μmol l^{-1} ATP or 5, 10 and 25 μmol l^{-1} 2-methylthio ATP (2-M ATP), (ii) 10, 50 and 100 μmol l^{-1} α,β-methylene ATP (α,β-Me ATP) or 1 μmol l^{-1} 2-methylthio ATP (2-M ATP), (iii) 10, 50 and 100 μmol l^{-1} β,γ-methylene ATP (β,γ-Me ATP), and (iv) 10, 50 and 100 μmol l^{-1} adenosine (Aden.) or 10, 50 and 100 μmol l^{-1} UTP. *Significantly ($P<0.05$) higher than the corresponding control value in the same culture period. † Significantly ($P<0.05$) lower than the corresponding control value in the same culture period.

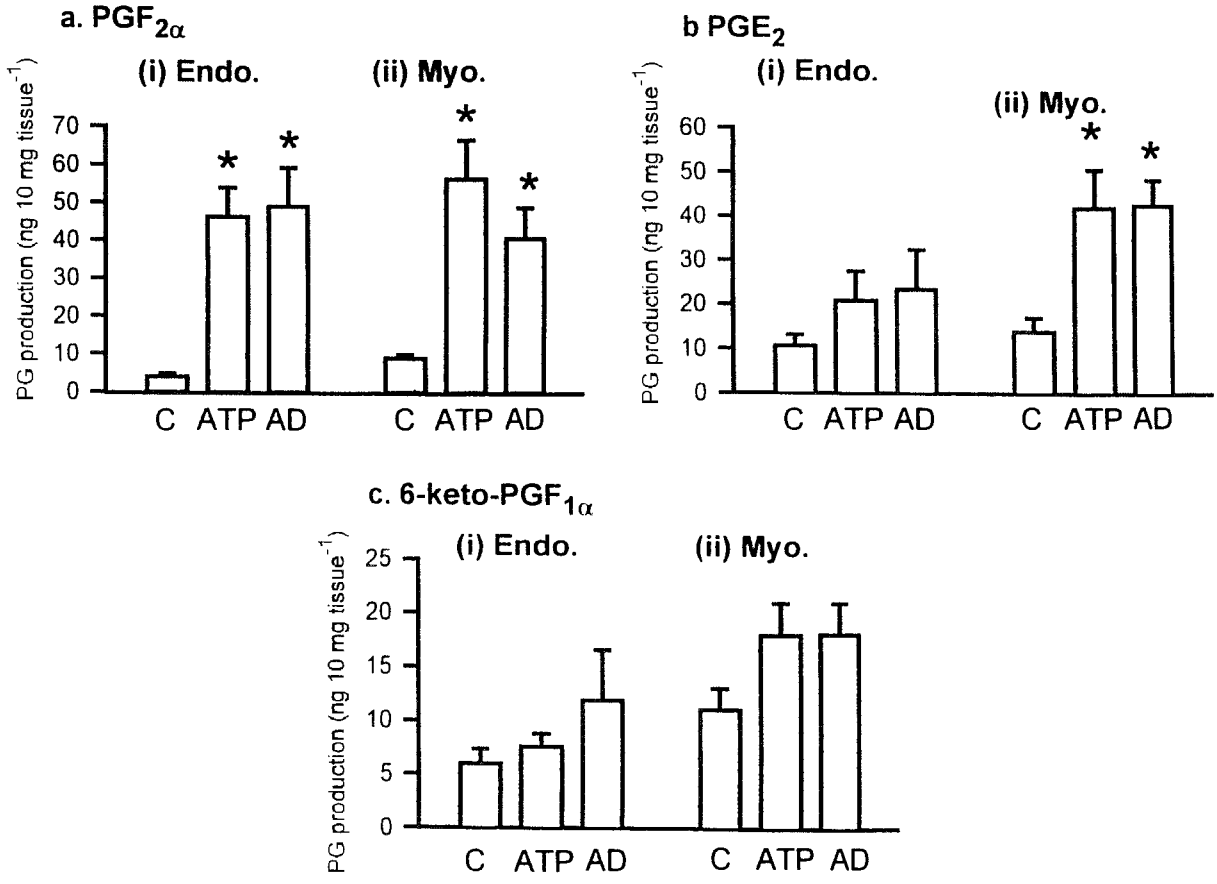


Figure 5 Mean (±s.e. mean, $n=5$) amounts of (a) prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), (b) PGE_2 and (c) 6-keto-$PGF_{1\alpha}$ produced during incubation of homogenates of (i) endometrium (Endo.) and (ii) myometrium (Myo.) following culture for 24 h in the absence (C) and presence of 100 μmol l^{-1} ATP or adenosine (AD). *Significantly ($P<0.05$) higher than the corresponding control (C) value.

uterus. Two other ATP derivatives (α,β-methylene ATP and β,γ-methylene ATP) failed to cause a significant increase in prostaglandin output from the superfused uterus, and thus raises doubts whether the spasmogenic actions of these two compounds on the guinea-pig uterus are mediated by prostaglandins. ATP, 2-methylthio ATP, α,β-methylene ATP, β,γ-methylene ATP, UTP and adenosine stimulate $PGF_{2\alpha}$ output from the guinea-pig endometrium and myometrium after 24 h of culture, although they exert a greater effect on the endometrium than the myometrium.

The technical assistance of Mrs Lorna Turnbull is greatly appreciated.

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(Received October 27, 2000
Accepted November 11, 2000)