Journal of Physiology (1989), **416**, *pp.* 623–643
With 9 text-figures
Printed in Great Britain

MEMBRANE CURRENTS ELICITED BY PROSTAGLANDINS, ATRIAL NATRIURETIC FACTOR AND OXYTOCIN IN FOLLICLE-ENCLOSED *XENOPUS* OOCYTES

BY R. MILEDI AND R. M. WOODWARD

From the Laboratory of Cellular and Molecular Neurobiology, Department of Psychobiology, University of California, Irvine, CA 92717, USA

(*Received 11 August 1988*)

SUMMARY

1 Membrane currents were recorded from voltage clamped *Xenopus laevis* oocytes, still surrounded by follicular cells, theca and enveloping inner ovarian epithelia (ovarian follicles).

2. Superfusing follicles with frog Ringer solution containing E-series prostaglandins (PGE_1 or PGE_2) or oxytocin (0·5–2 μM) generated slow membrane currents arising from an increase in membrane conductance to K^+.

3. Follicles taken from different frogs varied greatly in responsiveness to PGE and oxytocin. For example, enclosed oocytes with good sensitivity to prostaglandins responded to 1 nM-PGE, whereas follicles from some frogs failed to respond at 5 μM.

4. Oocytes with good responsiveness to PGE also produced K^+ currents to PGA_1, PGA_2, PGB_1, 11-deoxy-PGE_1 and 11-β-PGE_2, whereas $PGF_{2\alpha}$, PGI_2, PGD_2 and 8-iso-PGE_1 generally failed to elicit membrane currents.

5. Responses to PGE and oxytocin were mimicked by the adenylate cyclase activator forskolin or by intraoocyte pressure injection of cyclic nucleotides. Responses were potentiated by the phosphodiesterase inhibitors theophylline and 3-isobutyl-1-methylxanthine (IBMX). In IBMX (0·5 mM), human atrial natriuretic factor (ANF) (10–60 nM) elicited a similar K^+ conductance. This all implied that cyclic nucleotides played a role in the receptor–channel coupling mechanism of these responses.

6. Defolliculating oocytes effectively abolished responses to prostaglandins, oxytocin and ANF, suggesting that the currents arise in follicular cells.

7. The responses of PGE, oxytocin and ANF thus resembled currents elicited by catecholamines, adenosine, gonadotrophins and vasoactive intestinal peptide (VIP). However, PGE, oxytocin and ANF responses were not blocked by catecholaminergic or purinergic antagonists. Moreover, when comparing follicles isolated from different frogs, the sensitivity to PGE and oxytocin varied independently of that to gonadotrophin or VIP. These experiments suggest that *Xenopus* ovarian follicles contain specific and distinct receptors for PGE, oxytocin and ANF.

8. Acetylcholine attenuated the cyclic nucleotide-mediated K^+ responses, including currents elicited by PGE, oxytocin and ANF. Attenuation was not dependent on, or mimicked by, activation of the inositol phosphate–diacylglycerol messenger

pathways located in the oocyte itself, nor was it appreciably blocked by loading follicle-enclosed oocytes with 0·1–1·5 mM-EGTA.

INTRODUCTION

Catecholamines and adenosine elicit slow membrane currents in follicle-enclosed *Xenopus* oocytes (stage V and VI; Dumont, 1972) (Kusano, Miledi & Stinnakre, 1977, 1982; Lotan, Dascal, Cohen & Lass, 1982). These currents are associated with an increase in membrane conductance and are carried primarily by K^+ (Kusano *et al.* 1977, 1982; Lotan *et al.* 1982). We later found that porcine vasoactive intestinal peptide (VIP) and mammalian gonadotrophins evoked similar K^+ currents (Woodward & Miledi, 1987*a*, *b*). Responses to the various neurotransmitters/hormones appear to be mediated by distinct receptors, which are all coupled to an intracellular messenger pathway involving cyclic nucleotides. The different receptors are all thought to stimulate adenylate cyclase, causing an increase in intracellular adenosine 3′,5′-cyclic monophosphate (cyclic AMP) which in turn, probably through activation of cyclic AMP-dependent protein kinase(s), regulates the gating of specific K^+ channels (Lotan, Dascal, Oron, Cohen & Lass, 1985; Van Renterghem, Penit-Soria & Stinnakre, 1985; Stinnakre & Van Renterghem, 1986; Smith, Brooker & Brooker, 1987; Woodward & Miledi, 1987*a*, *b*). These currents are all essentially abolished by defolliculation, suggesting that the responses are located in follicular cells (follicle cells) (e.g. Kusano *et al.* 1982; Smith *et al.* 1987; and accompanying paper, Miledi & Woodward, 1989). Thus, when recording from within follicle-enclosed oocytes, cyclic nucleotide-activated K^+ currents are only detected because follicular cells are electrically coupled to the oocyte by gap junctions (Browne, Wiley & Dumont, 1979; Browne & Werner, 1984; van den Hoef, Dictus, Hage & Bluemink, 1984; authors' unpublished results).

Acetylcholine (ACh) acting through muscarinic receptors can elicit complex electrical responses, consisting of slow oscillatory Cl^- currents sometimes superimposed over an increase in K^+ conductance (e.g. Kusano *et al.* 1977, 1982; Dascal, Lotan & Lass, 1987). The oscillatory Cl^- currents arise largely or wholly in the oocyte itself (e.g. Kusano *et al.* 1982; Miledi & Woodward, 1989). These Cl^- currents are generated through an intracellular pathway involving hydrolysis of inositol phospholipids, mobilization of intracellular Ca^{2+} by inositol phosphates and activation of Ca^{2+}-gated Cl^- channels in the oocyte membrane (e.g. Miledi, 1982; Miledi & Parker, 1984; Oron, Dascal, Nadler & Lupu, 1985; Parker & Miledi, 1986). In addition, this pathway appears to be activated by a proteinaceous factor in serum (G. Tigyi, C. M. Matute, D. L. Dyer & R. Miledi, unpublished results), and in some oocytes by extracellular divalent cations (e.g. Cd^{2+}, Zn^{2+} and Co^{2+}; Miledi, Parker & Woodward, 1988, 1989). Acetylcholine also has the effect of attenuating K^+ currents elicited by hormones/neurotransmitters, forskolin and intraoocyte injections of cyclic AMP (Dascal, Lotan, Gillo, Lester & Lass, 1985; Van Renterghem *et al.* 1985; Stinnakre & Van Renterghem, 1986; Woodward & Miledi, 1987*a*, *b*). This effect seems to be independent of cyclic nucleotide levels and is mimicked by bath-applied phorbol esters (Dascal *et al.* 1985).

When comparing the structure and physiology of mammalian and *Xenopus* ovaries, we noticed an apparent analogy between receptor systems coupled to

adenylate cyclase in mammalian granulosa–cumulus cells, and receptors which elicited K^+ currents in follicle-enclosed *Xenopus* oocytes. For example, rat granulosa cells can carry β-adrenergic, gonadotrophin and VIP receptors, all of which couple to cyclic AMP synthesis (e.g. Adashi & Hsueh, 1981; Kliachko & Zor, 1981; Hsueh, Jones, Adashi, Wang, Zhuang & Welsh, 1983; Davoren & Hsueh, 1985). Pursuing the analogy, we noted that prostaglandin E_2 (PGE_2), which is implicated in various ovarian processes (e.g. Behrman, 1979; Armstrong, 1981; Espey, Norris & Saphire, 1986), had also been shown to raise cyclic AMP levels in cultured granulosa cells (Kolena & Channing, 1972; Goff & Armstrong, 1983). Furthermore, atrial natriuretic factor (ANF) was recently found to stimulate guanylate cyclase activity in rat luteal cells (Budnik, Brunswig & Mukhopadhyay, 1987) and human granulosa–lutein cells (Pandey, Osteen & Inagami, 1987). If E-series prostaglandins and ANF had similar activities in *Xenopus* ovarian follicles, we reasoned it might be possible to detect these receptors by electrical recording.

The present paper describes membrane current responses elicited by PGE, ANF and oxytocin in *Xenopus* oocytes and discusses possible mechanisms by which ACh attenuates cyclic nucleotide-activated K^+ currents.

METHODS

Experiments were started at University College London, and were completed at the University of California at Irvine. Follicles (follicle-enclosed oocytes) were plucked from the ovaries of laboratory-reared adult *Xenopus laevis*, obtained from Xenopus Ltd (Surrey, UK), Xenopus I (Ann Arbor, MI, USA) and Nasco (Fort Atkinson, WI, USA) and killed by decapitation. Electrical recordings were made at room temperature (22–25 °C) in a bath (*ca* 1 ml) continuously perfused (5–10 ml min^{-1}) with normal frog Ringer solution (in mM): NaCl, 115; KCl, 2; $CaCl_2$, 1·8; HEPES (*N*-2-hydroxyethylpiperazone-*N*-2-ethanesulphonic acid), 5; pH 7·0 (for further details see Kusano *et al.* 1982; Miledi, 1982). Except where indicated, membrane currents were recorded by voltage clamping oocytes (stage V or VI; Dumont, 1972) still surrounded by their enveloping ovarian tissues. Follicle-enclosed oocytes were stored at 16 °C in sterile Barth's medium (in mM): NaCl, 88; KCl, 1; $NaHCO_3$, 2·4; $MgSO_4$, 0·82; $Ca(NO_3)_2$, 0·33; $CaCl_2$, 0·41; HEPES, 5; pH to 7·4 with NaOH, usually with nyastatin (50 U ml^{-1}) and gentamycin (0·1 mg ml^{-1}) for use over the first 4 days of storage.

Prostaglandins PGE_1 and PGE_2 were purchased from Cayman Chemical (Ann Arbor, MI, USA), Calbiochem or Sigma; PGA_1, PGA_2, PGB_1, PGD_2 and $PGF_{2\alpha}$ from Sigma; 11-deoxy-PGE_1, 11-β-PGE_2, 8-iso-PGE_1 and prostacyclin (PGI_2) from Cayman. Prostaglandins were initially dissolved as concentrated stocks in ethanol (EtOH) or dimethyl sulphoxide (DMSO) at 1–10 mg ml^{-1} and stored at −20 °C. PGI_2 was dissolved at 1 mg ml^{-1} in a 20 mM-Trizma buffer (pH 10), stored at 4 °C and used for about 1 week. To counter any instability of these compounds, dilute Ringer solutions of prostaglandins were made up within 2 min of application to follicles; this was reduced to *ca* 30 s in the case of PGI_2. Human ANF (twenty-eight amino acid), oxytocin, a crude pituitary preparation of porcine follicle stimulating hormone (pFSH) and forskolin (*Coleus forskohlii*) were obtained from Calbiochem. Stock ANF was made up at 100 μg ml^{-1} in 10 mM-HEPES (pH 7·0) and oxytocin at 12 mg ml^{-1} in 10 mM-HEPES (pH 7·0); both were aliquoted and stored at −20 °C. SCH 23390 was from Research Biochemicals, Inc. (Wayland, MA, USA) and rabbit serum (T100) from Diagnostic Biochemicals, Inc. (San Diego, CA, USA). All other drugs and reagents were purchased from Sigma.

Intraoocyte injections were by pneumatic pressure ejection from micropipettes (see Miledi & Parker, 1984). Injection pipettes were loaded with filtered solutions of either 10 mM-guanosine 3′,5′-cyclic monophosphate (cyclic GMP), 1–10 mM-cyclic AMP or 50 mM-EGTA (ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*′-tetraacetic acid), all in 10 mM-HEPES, pH adjusted to 7·0 with KOH. The pressure regulator was set at 200 kPa and the length of the pulse (50–2000 ms) selected to eject droplets of 100–200 pl. Defolliculation was carried out either enzymatically with

collagenase (Kusano *et al.* 1982) or by manual removal of inner ovarian epithelia and rolling oocytes on poly-L-lysine-treated slides (see accompanying paper, Miledi & Woodward, 1989).

RESULTS

Membrane currents elicited by prostaglandins

Ovarian follicles were routinely screened for membrane responses to prostaglandins while clamping enclosed oocytes at −60 mV (see Kusano *et al.* 1982). Prostaglandins were applied to over 235 follicles from fifty-three frogs, and > 80% responded to PGE_1 or PGE_2 with an outward current, associated with an increase in membrane conductance (Fig. 1*A*). As with other native responses, sensitivity to PGE varied greatly when comparing follicles from different frogs. For example, in the most sensitive follicles outward currents were detected with 1–10 nM-PGE_1, and application of 1·0 μM-PGE_1 elicited responses of > 400 nA. At the other extreme, follicles from some frogs completely failed to respond to PGE_1 or PGE_2 at concentrations as high as 5·0 μM. To estimate the prevalence of prostaglandin responses two to ten follicles from each of the fifty-three frogs used in this study were tested for responses to 1 μM-PGE. Follicles from six frogs had no detectable response, those from thirty-five frogs had mean responses between 2–50 nA and those from twelve frogs gave mean currents in the range 50–500 nA.

Depending on follicle sensitivity and concentration of PGE_1 or PGE_2, response latencies varied between 15 and 60 s, with rising phases of 0·5–3·0 min. Extended exposure typically resulted in desensitization (Fig. 1*A*), though the rate at which this occurred again varied between follicles from different frogs. Currents elicited by PGEs tended to decline or disappear upon repeated exposures; however, in follicles

---

Fig. 1. *A*, membrane currents elicited by PGE_2. Follicle-enclosed oocyte, voltage clamped at −60 mV with clamp potential periodically stepped to −50 mV to monitor changes in membrane conductance. The follicle was continuously superfused with frog Ringer solution. Exposures to PGE_2 indicated by bars; dead time for the solution change was *ca* 20 s. Exposure to 1 μM-PGE_2 was prolonged to show desensitization of response. Unless otherwise stated these recording conditions were used in all following figures; outward current is denoted by upward deflection. Capacitative transients on the steps in command potential have been touched out. *B* and *C*, current–voltage relationships of drug-induced currents. *B*, a single follicle-enclosed oocyte was exposed to 100 nM-PGE_2 and the voltage dependence of this current determined (▽). After washing out the PGE_2 the follicle was pre-incubated with 0·5 mM-3-isobutyl-1-methylxanthine (IBMX) (which in this case failed to generate any significant membrane conductance), re-exposed to 100 nM-PGE_2 in IBMX and the voltage dependence of this amplified current established (▲). The follicle was washed for 15 min and exposed to 100 nM-forskolin alone; the *I–V* curve of this response was likewise determined (■). Final resting potential = −52 mV; upon withdrawal the voltage-monitoring electrode had a tip potential of −1 mV. *C*, comparison of *I–V* curves, determined sequentially in a single follicle (same frog as *A*), for membrane currents elicited by 1 μM-PGE_2 (▽), *ca* 2 pmol cyclic AMP injected into oocyte (■), 0·5 μM-forskolin (□) and 10 μM-adenosine (○). Injection pipette (cyclic AMP) inserted at start of experiment and time allowed for resealing of membranes before evoking any responses. Final resting potential = −49 mV and upon withdrawal voltage-monitoring electrode carried a tip potential of +3 mV.

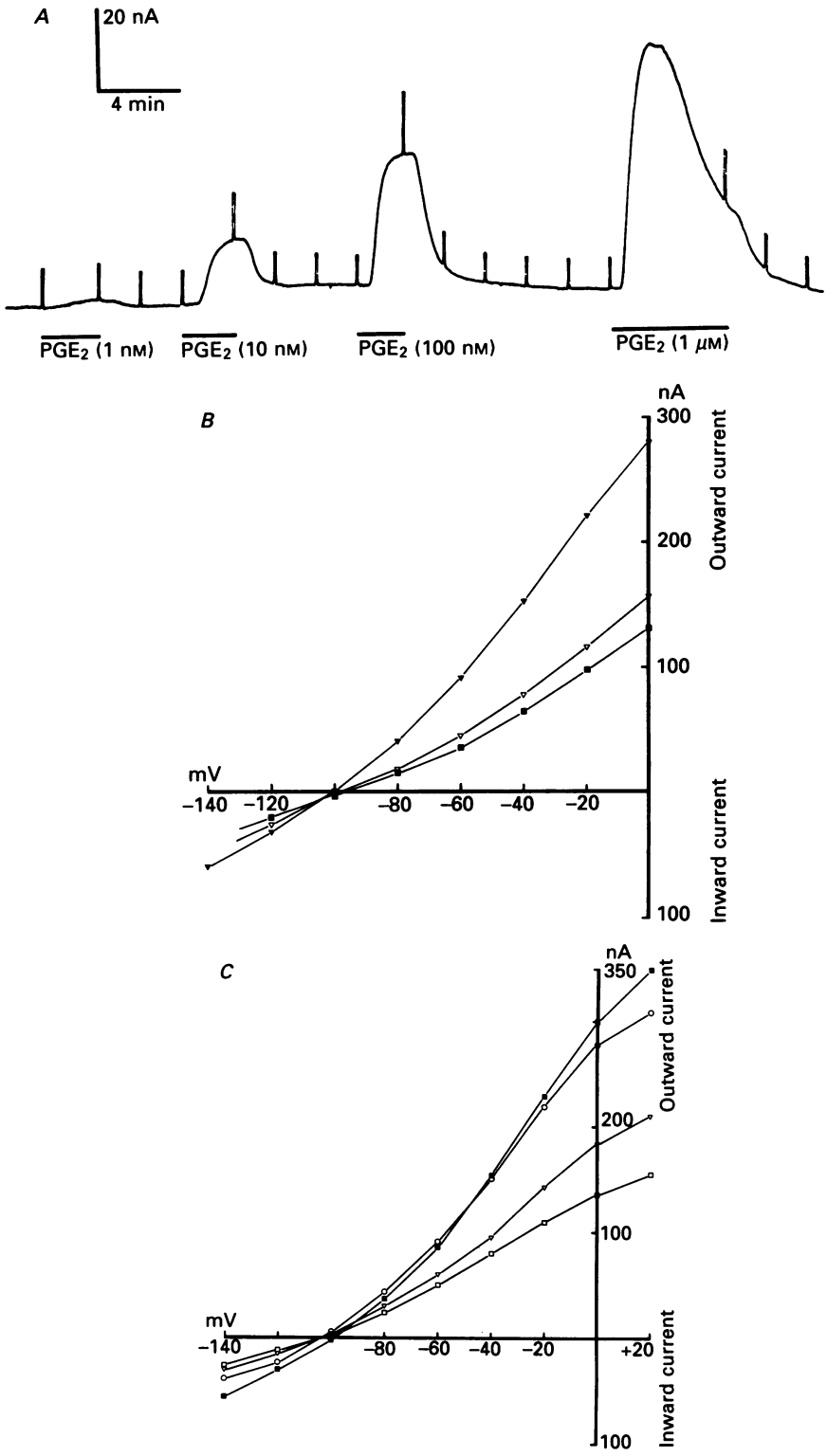

Fig. 1. For legend see facing page.

with good sensitivity, three or four reproducible responses could be obtained by using submaximal concentrations of PGEs (e.g. 50 nM) applied at intervals of 20 min. There was no obvious difference in potency between PGE_1 and PGE_2 when tested on the same follicle, so the abbreviation PGE will be used to indicate either PGE_1 or PGE_2. Neither DMSO, at 0·1 μl ml^{-1} (1·4 mM), nor ethanol at 1 μl ml^{-1} (17 mM) elicited any significant outward currents. Occasionally, follicles from some frogs responded to vehicle with small (< 3 nA) inward or outward currents, and high concentrations of ethanol tended to intensify any spontaneous oscillatory Cl^- currents.

In general, sensitivity to PGE declined over the first week that follicles were stored in Barth's medium. But we often noticed (sixteen frogs) that freshly taken follicles initially had low sensitivity to PGE which then improved over the first 10–20 h storage. This effect was not dependent on the presence of antibiotics in Barth's medium. Screening follicles for PGE sensitivity solely on day of isolation would therefore tend to underestimate the prevalence of this response. Like currents evoked by β-adrenergic agonists, dopamine, adenosine, VIP and gonadotrophins, the currents elicited by PGE were effectively abolished by defolliculation (see accompanying paper, Miledi & Woodward, 1989).

Ionic basis of the prostaglandin response

Similarities between responses to PGE and other native responses (e.g. catecholamine, adenosine and gonadotrophin) suggested that PGE currents were likewise carried by K^+. This we confirmed by examining the voltage dependence of PGE currents, determined by briefly stepping clamp potentials to different voltages during PGE responses, and subtracting the current pulses recorded in resting membranes. The *I–V* curves were fairly linear over the range −120 to +20 mV (e.g. Fig. 1*B*), reversed at *ca* −100 mV in normal frog Ringer solution (the predicted reversal for K^+ in *Xenopus* oocytes; see Kusano *et al.* 1982), and were largely indistinguishable from currents elicited by gonadotrophins, VIP or adenosine (Fig. 1*C*) in the same follicle. Like these responses, PGE currents were abolished by 2 mM-$BaCl_2$ and were substantially blocked by 20 mM-tetraethylammonium bromide.

Since the ionic basis of PGE responses was similar to K^+ currents elicited by β-adrenergic agonists, dopamine, adenosine, gonadotrophins and VIP, it was necessary to determine whether PGE responses were due to activation of any of these receptors, either via a direct interaction, or by PGE causing release of these agonists from the follicle. Both possibilities appear unlikely because: (1) PGE responses were largely unaffected by antagonists which abolished the follicle's responses to noradrenaline, adenosine, dopamine and ACh (e.g. Fig. 2) and (2), though we had no specific antagonists to distinguish PGE responses from those to gonadotrophins or VIP, it was clear, when comparing follicles from different frogs, that there were large and independent variations in sensitivity to these agonists (e.g. Fig. 3). The receptor antagonists used in (1) were: 2 μM-timolol, β-adrenergic; 0·1 mM-theophylline, P1-purinergic (Lotan *et al.* 1982); 2 M-SCH 23390, D1-dopaminergic; 10 mM-atropine, muscarinic. Timolol was used in preference to propranolol as a β-adrenergic antagonist because in some cases > 10 μM-propranolol suppressed forskolin responses, indicating that propranolol inhibited K^+ currents in some 'non-specific' manner independent of its action on β-adrenergic receptors.

Our experiments therefore implied that there was a specific and distinct membrane current response to PGE. We then compared the potency of PGE with other classes of prostaglandins. Follicles with good sensitivity to PGE also elicited K^+ currents in response to PGA_1, PGA_2 and in some cases PGB_1 (0·5–5·0 μM); however, these

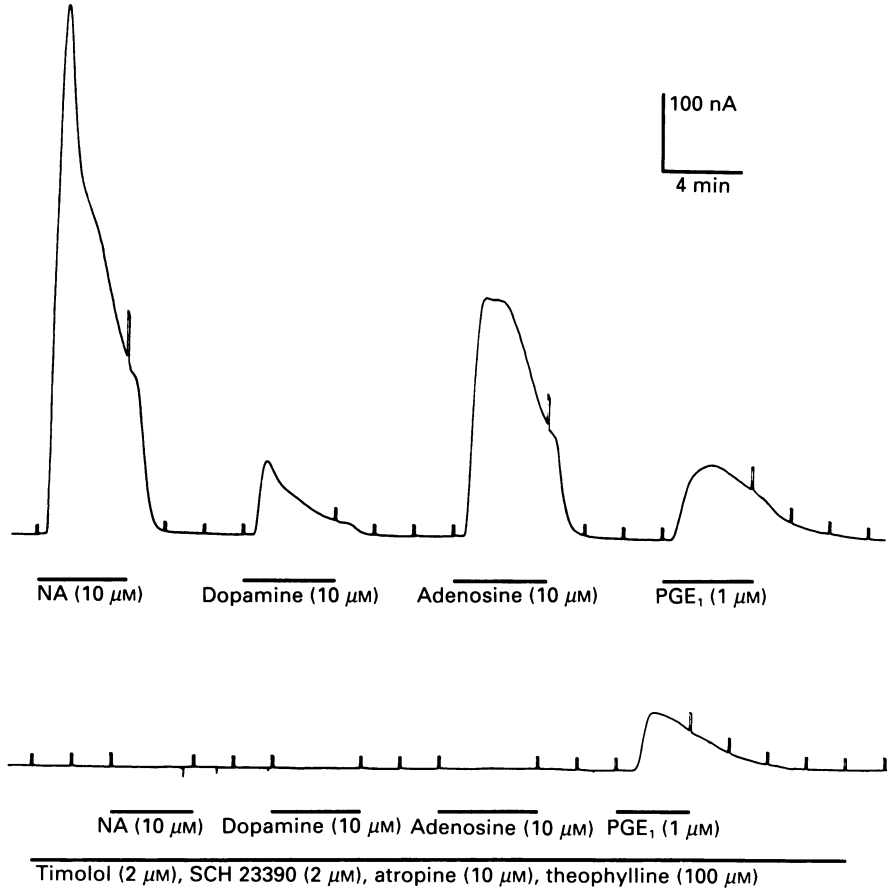

Fig. 2. Preservation of response to PGE_1 in a mixture of receptor antagonists. Upper records: a single follicle-enclosed oocyte was exposed to noradrenaline (NA), dopamine, adenosine and PGE_1, demonstrating sensitivity to all these agonists. Lower records: the follicle was then superfused with Ringer solution containing a 'cocktail' of receptor antagonists, as indicated, and re-exposed to the same agonists. K^+ currents generated by noradrenaline, dopamine and adenosine were abolished, whereas the response to PGE_1 was largely preserved. (See text for details.)

currents were always small compared to those elicited by PGE in the same follicle. For example, in one frog the response of five follicles to 1·0 μM-PGA_1 was 15 ± 6 nA, whereas the same follicle's subsequent response to 1·0 μM-PGE_1 was 55 ± 29 nA. Occasionally PGB_1 elicited small (< 5 nA), maintained inward currents (e.g. Fig. 5), but these were only seen at concentrations of 2–5 μM and have not been investigated further. In contrast, PGD_2, $PGF_{2\alpha}$ and PGI_2, when applied at concentrations up to 5·0 μM, failed to generate any significant membrane currents. We also compared the follicle's sensitivity to various PGE analogues, which gave the following sequence:

$$\text{PGEs} > \text{11-deoxy-PGE}_1 > \text{11-}\beta\text{-PGE}_2 > \text{8-iso-PGE}_1.$$

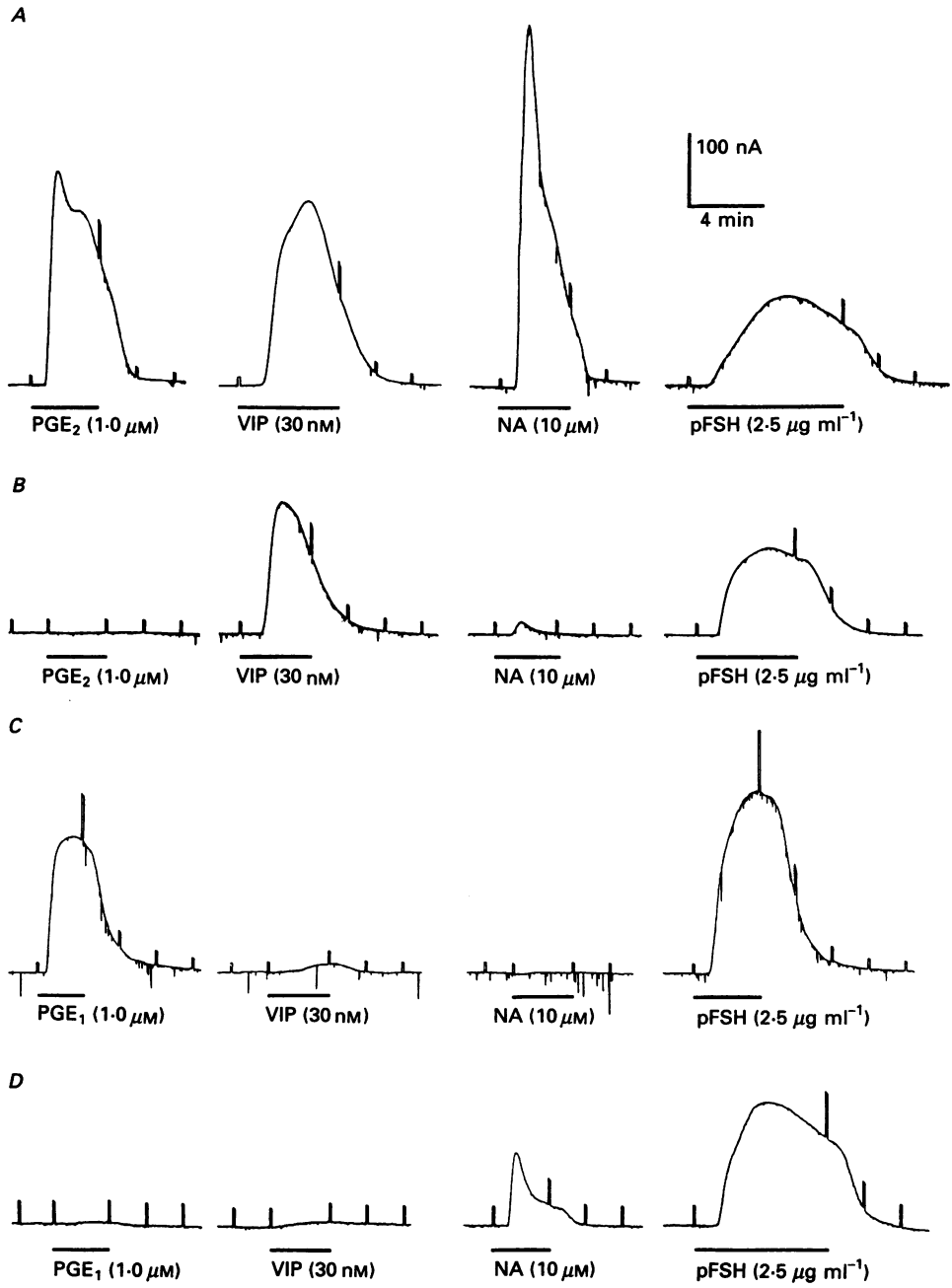

Fig. 3. *A–D*, independence of responses seen in different oocytes. Four follicle-enclosed oocytes, taken from four different frogs, were exposed to various agonists (VIP, vasoactive intestinal peptide; pFSH, porcine follicle stimulating hormone) at intervals of 8–12 min. Small downward deflections are spontaneous oscillatory Cl^- currents present in some follicles, particularly (C). As indicated, sensitivity to PGE, VIP and noradrenaline appeared to vary independently between different frogs, whereas responses to pFSH were less variable and also tended to desensitize more slowly. Generally, currents elicited by 1 μM-PGE were small when compared with responses to 2·5 μg ml^{-1} pFSH in the same follicle.

Currents elicited by 11-deoxy-PGE_1 were 10–15% and by 11-β-PGE_2 0–2% of subsequent responses to PGE (eleven follicles, four frogs); 8-iso-PGE_1 was inactive up to 5·0 μM. It should be noted that commercially available preparations of PGAs and PGBs suffer from cross-contamination, and that on prolonged storage 8-iso-PGE_1 did acquire some activity, probably a consequence of racemization.

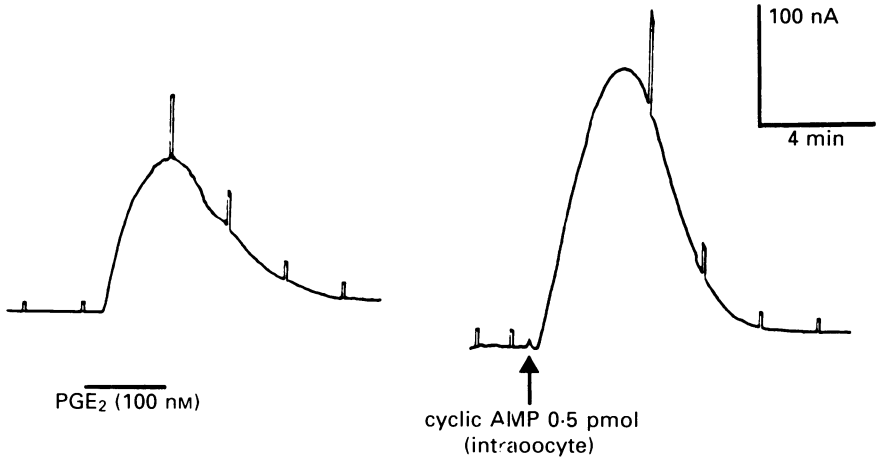

Fig. 4. Comparison of membrane responses elicited by PGE_2 and by intraoocyte pressure injection of cyclic AMP. A follicle-enclosed oocyte was voltage clamped as in Fig. 1 and exposed to PGE_2 as indicated. An injection pipette (containing 1 mM-cyclic AMP) was then inserted into the oocyte (not shown), causing loss of input resistance. A lapse of 10 min was allowed for some resealing of membranes and a return to stable recording conditions whereupon the oocyte was injected with *ca* 0·5 pmol cyclic AMP (arrow). The small outward spike upon injection was also seen when injecting 10 mM-HEPES.

The prostaglandin response and cyclic nucleotides

As described, cyclic AMP is implicated in mediating the K^+ currents elicited by β-adrenergic agonists, dopamine, adenosine, gonadotrophin and VIP (e.g. Lotan *et al.* 1985; Van Renterghem *et al.* 1985; Woodward & Miledi, 1987*a*, *b*). Like these responses, K^+ currents elicited by prostaglandins were mimicked in the same follicle by the adenylate cyclase activator forskolin (Fig. 1*B* and *C*) or by intraoocyte injection of cyclic AMP (Figs. 1*C* and 4). Furthermore, prostaglandin responses were consistently potentiated by the phosphodiesterase blockers 3-isobutyl-1-methyl-xanthine (IBMX, 0·5 mM; Fig. 5) or theophylline (2–5 mM). For example, six follicles from one frog elicited K^+ currents of 15 ± 14 nA to 0·5 μM-PGE_1, whereas after 8 min pre-incubation in 0·5 mM-IBMX, the same concentration of PGE_1 elicited responses of 57 ± 42 nA, degrees of amplification ranging between 2- and 20-fold. Determining the voltage dependence of both control and IBMX-potentiated currents showed that amplified PGE responses were still carried by K^+ and had not been significantly contaminated by other conductances (Fig. 1*B*). With IBMX it was also possible to elicit responses at concentrations of PGE which were subthreshold in control exposures.

Potassium currents generated by PGAs, PGB_1, 11-deoxy-PGE_1 and 11-β-PGE_2

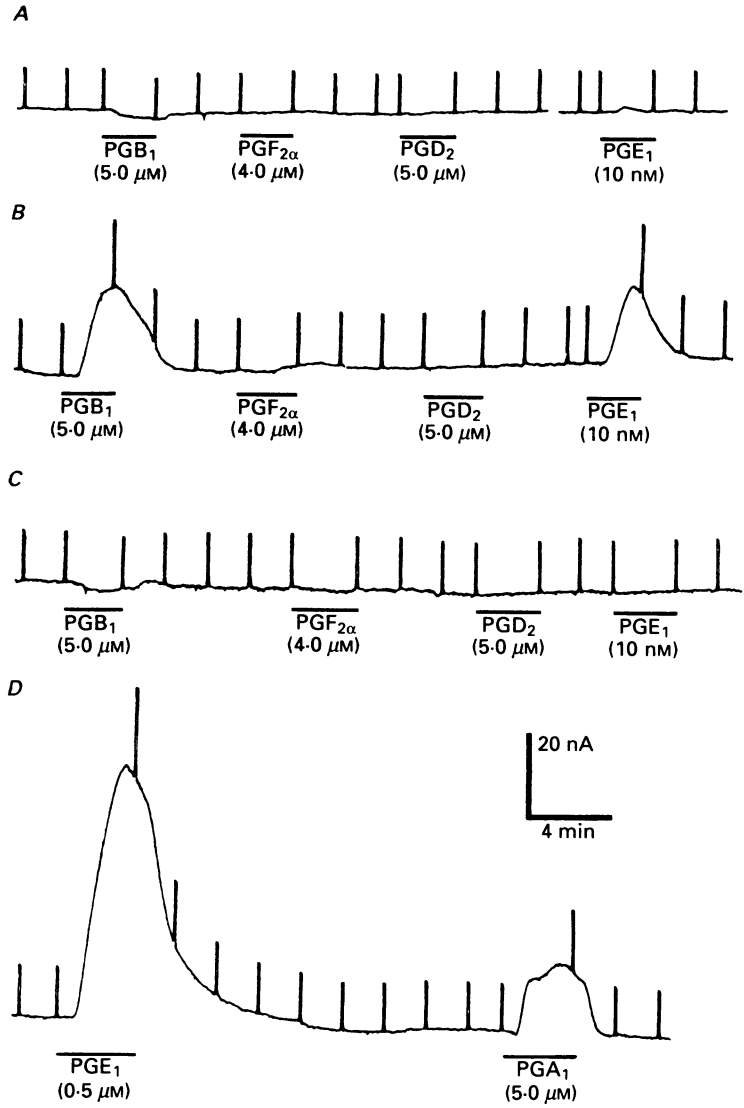

Fig. 5. Relative potencies of different prostaglandins, and potentiation of responses by IBMX. *A*, a single follicle was exposed first to PGE_1 (out of sequence) then to PGB_1, $PGF_{2\alpha}$ and PGD_2 as indicated. At concentrations indicated PGE_1 elicited a small outward current and PGB_1 a small maintained inward current, whereas $PGF_{2\alpha}$ and PGD_2 failed to generate responses. The follicle was then incubated in 0·5 mM-IBMX which elicited a transient K^+ current (*ca* 60 nA) that was allowed to desensitize (not shown). *B*, follicle re-exposed to prostaglandins in presence of IBMX; both PGB_1 and PGE_1 generated outward currents. *C*, the follicle was then washed for 15 min and again exposed to prostaglandins which failed to generate outward currents. In *D*, however, the follicle did subsequently respond to an increased concentration of PGE_1 and to PGA_1.

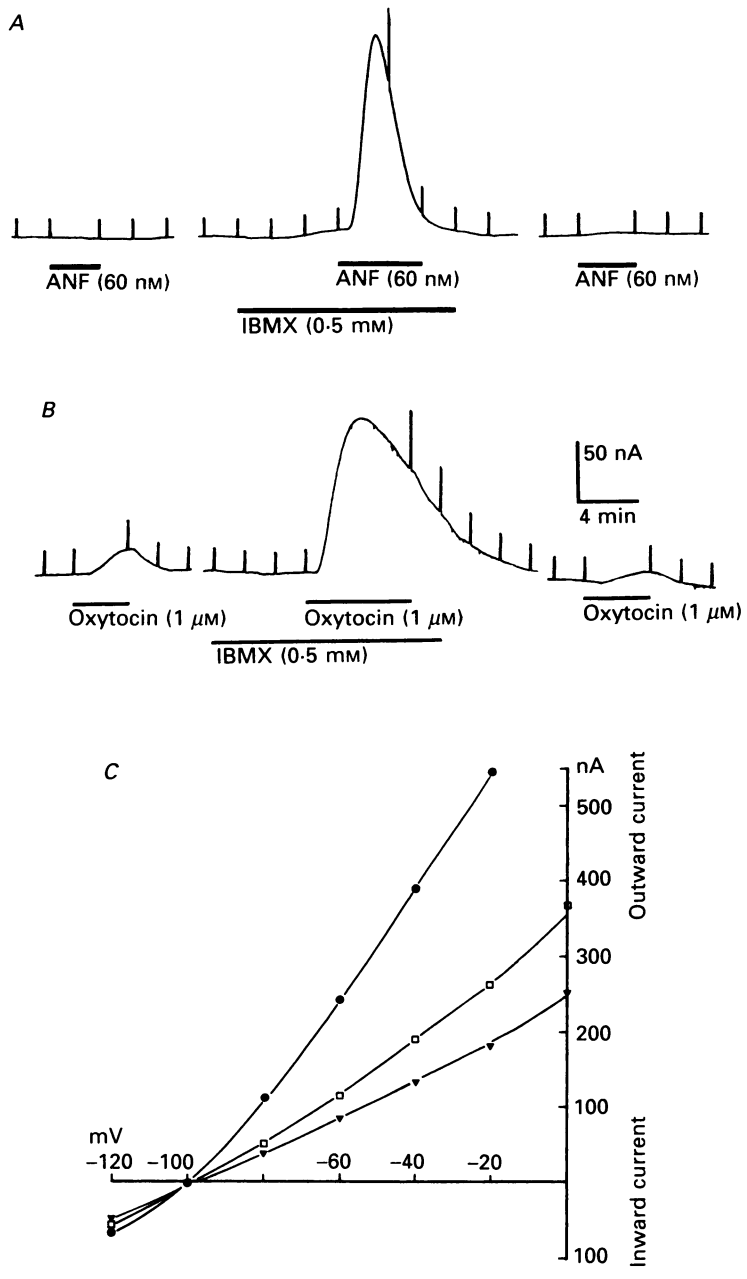

Fig. 6. *A*, membrane current to ANF revealed following a short pre-incubation in IBMX. The initial exposure to ANF fails to elicit a response, whereas in the presence of IBMX ANF develops a substantial outward current which desensitizes upon prolonged exposure (15 and 30 min was between records). *B*, potentiation of the membrane current to 1 μM-oxytocin by IBMX. *C*, current–voltage relationships. The current elicited by 60 nM-ANF in 0·5 mM-IBMX (●). In this follicle IBMX alone failed to elicit any significant current and the ANF response desensitized more slowly than in (*A*). Current evoked by 1 μM-oxytocin (▼) and for comparison the current evoked by 0·5 μM-forskolin in the same follicle (□).

were also potentiated by phosphodiesterase inhibitors, whereas PGD_2 and 8-iso-PGE_1 remained inactive. Particularly sensitive follicles sometimes gave small K^+ responses to 5·0 μM-$PGF_{2\alpha}$ and PGI_2. However, in these follicles phosphodiesterase inhibitors allowed PGE responses to be detected at concentrations as low as 100 pM

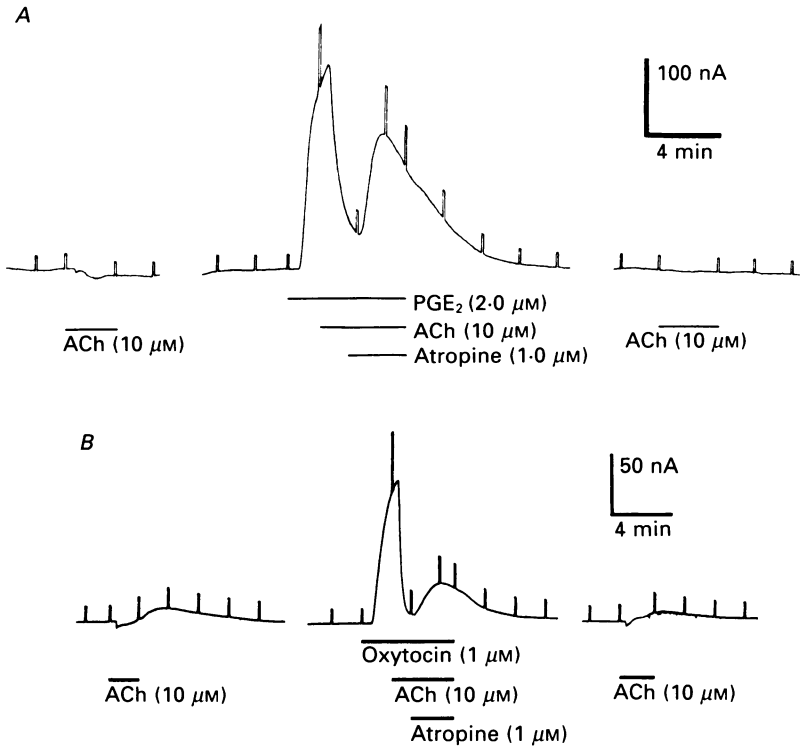

Fig. 7. *A*, inhibition of an established PGE_2 response by acetylcholine. A follicle with good sensitivity to PGE_2 was first exposed to 10 μM-ACh which elicited a small biphasic current with little change in membrane conductance. After washing for 20 min the follicle was exposed to 2·0 μM-PGE_2 producing a substantial K^+ current. Application of ACh during this response rapidly attenuated the conductance, an effect partly reversed by atropine. The follicle was washed for a further 15 min (break in record), and re-exposed to ACh which failed to elicit appreciable membrane currents, either due to refractoriness or to residual antagonism by atropine. Dead time for the solution change was *ca* 15 s. *B*, reduction of an established oxytocin response by ACh; experiment as in (*A*), with 22 and 35 min wash in breaks between records.

and given this sensitivity we cannot be certain that currents generated by $PGF_{2\alpha}$ or PGI_2 were not due to low-level contamination (*ca* 0·005%) with 'active' prostaglandins.

In all cases (thirty-three follicles, twelve frogs), sensitivity to PGAs and PGB_1 was lower than, but invariably paralleled, that of PGE. This was similarly true for responses to 11-deoxy-PGE_1 and 11-β-PGE_2. The membrane current activity of DMSO and ethanol was rechecked in the presence of phosphodiesterase inhibitors and was again insignificant (see above). In some experiments we independently

varied concentrations of PGE and vehicle (DMSO or ethanol) and found that the size of K^+ responses depended solely on concentrations of PGE.

Membrane currents elicited by ANF and oxytocin

Following the procedure described for prostaglandins, ANF was tested on thirty-five follicles from fourteen frogs. Though ANF elicited no significant membrane

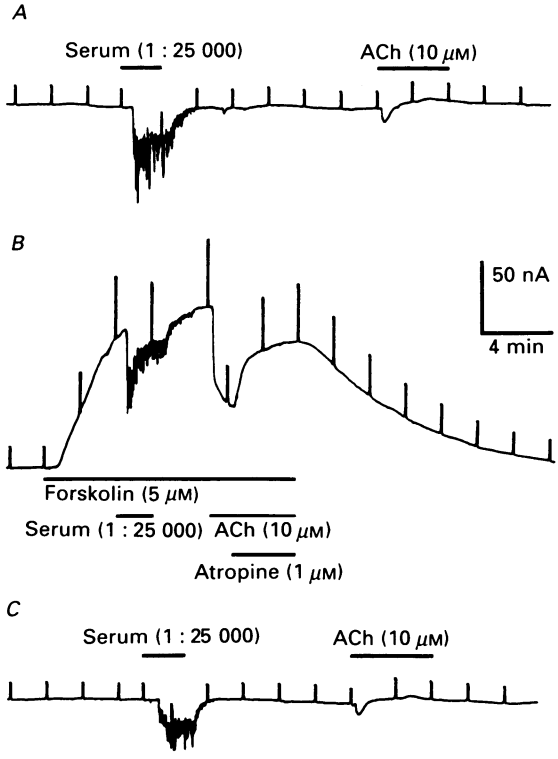

Fig. 8. Comparing effects of rabbit serum and ACh on the K^+ current elicited by forskolin. *A*, currents elicited by control exposures to serum and ACh in an 'epithelium-free' oocyte. Serum elicits an oscillatory Cl^- response, whereas ACh evokes a small, transient Cl^- current followed by a slight outward current typically carried by K^+. Between records the oocyte was washed for 25 min. *B*, exposures to serum and ACh were repeated superimposed on a K^+ current elicited by forskolin. Serum again elicited the oscillatory Cl^- response but did not cause any appreciable suppression of K^+ current. In contrast, a 60 s exposure to ACh attenuated the forskolin response to *ca* 40% of the established current. Oocyte was then washed for 20 min. *C*, repeating controls, serum response showed some desensitization.

currents when applied alone, after pre-incubating follicles in 0·5 mM-IBMX, 60 nM-ANF evoked outward currents ranging between 10 and 525 nA in twenty-two follicles from eight of the frogs tested (Fig. 6*A*). Follicles with highest sensitivity responded to ANF at concentrations between 5–10 nM. The current was again associated with an increase in membrane conductance, reversed at *ca* −100 mV (Fig. 6*C*) and was completely blocked by 2 mM-$BaCl_2$. This again indicated that the current consisted of an increase in conductance to K^+. The ANF responses were not

abolished by β-adrenergic, muscarinic, dopaminergic or purinergic receptor antagonists (as described above) and consistently desensitized more rapidly than currents evoked by gonadotrophins.

In separate experiments we found that 0·5–2 μM-oxytocin also elicited outward currents associated with an increase in membrane conductance (fifteen follicles, four frogs). In all respects this current appears to be the same as those elicited by

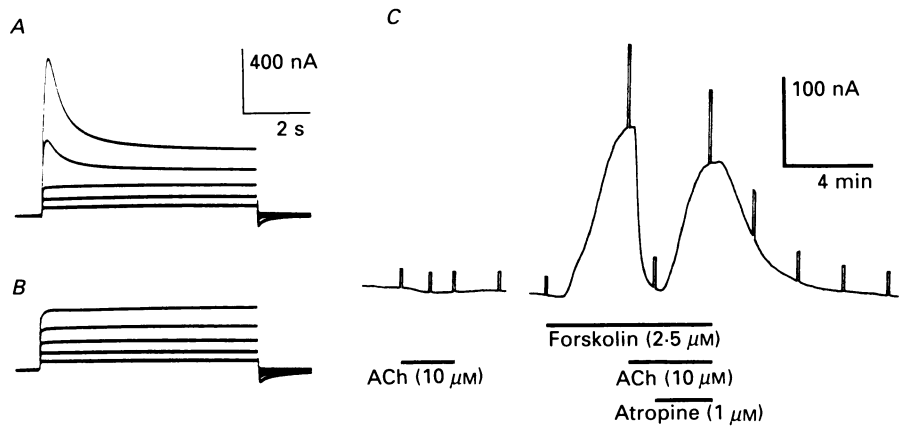

Fig. 9. Suppression of K^+ currents by ACh is not blocked by loading oocytes with EGTA. *A*, epithelium-free oocyte voltage clamped at −80 mV and bathed in Ringer solution with 10 mM-$CaCl_2$. Currents evoked by depolarizing pulses to −60, −40, −20, 0 and +20 mV; transient outward currents apparent at 0 and +20 mV. *B*, same voltage pulses 5 min after injecting *ca* 200 pmol EGTA; transient outward current abolished. Oocyte was then bathed in normal Ringer solution for *ca* 60 min. *C*, following injections with EGTA, ACh still strongly attenuated K^+ responses elicited by forskolin; in this case the current was reduced by > 90% (see text).

catecholamines, adenosine, gonadotrophins, VIP and prostaglandins. Oxytocin currents reversed at *ca* −100 mV (Fig. 6*C*), were blocked by 2 mM-$BaCl_2$ and were potentiated by phosphodiesterase inhibitors (Fig. 6*B*). Like PGE and ANF responses, currents elicited by oxytocin were substantially preserved in a mixture of receptor antagonists which had abolished responses to noradrenaline, dopamine, adenosine and ACh (as above).

As expected, K^+ currents elicited by both ANF and oxytocin were effectively abolished by defolliculation. For example, five follicles from one frog gave a current of 233 ± 60 nA to 2 μM-oxytocin, whereas five manually defolliculated oocytes gave no detectable responses (see Miledi & Woodward, 1989).

Attenuation of K^+ currents by acetylcholine

As described, ACh acting upon muscarinic receptors attenuates the cyclic nucleotide-mediated K^+ currents elicited by noradrenaline, dopamine, adenosine, gonadotrophins, VIP, forskolin and intraoocyte injections of cyclic AMP (e.g. Dascal *et al.* 1985; Van Renterghem *et al.* 1985; Stinnakre & Van Renterghem, 1986; Woodward & Miledi, 1987*a*, *b*). Responses to PG, ANF and oxytocin were likewise reduced by ACh (e.g. Fig. 7); this effect occurred whether ACh was applied to follicles before or during K^+ responses.

Attenuation of K^+ currents is mimicked by phorbol esters (Dascal *et al.* 1985 authors' unpublished results), which suggests that the effect is mediated through diacylglycerol (DG) and activation of protein kinase C (C-kinase). Defolliculation experiments clearly suggested that cyclic nucleotide-activated K^+ currents are located in the follicular cells (see accompanying paper, Miledi & Woodward, 1989). We therefore investigated whether the oocyte's phosphatidylinositol–Ca^{2+} messenger cascade played a role in suppressing K^+ responses, using oscillatory Cl^- currents as an electrical assay for activation of this pathway in the oocyte itself.

Firstly, experiments on > 200 follicle-enclosed oocytes (*ca* seventy frogs) showed that attenuation of cyclic nucleotide-activated K^+ currents by ACh was almost invariably present, though there were significant differences in potency when comparing oocytes taken from different frogs. For some oocytes, forskolin responses of 200–600 nA were completely abolished by 0·5 μM-ACh, while in others 100 μM-ACh reduced these responses by only 20–40%. When comparing oocytes, there was also no element of the ACh response which obviously correlated with the potency of attenuation. For example, in four oocytes from one frog, 10 μM-ACh itself elicited no detectable membrane currents (< 1 nA), but nevertheless 1 μM-ACh completely suppressed responses to forskolin. Moreover, in oocytes where 10–100 μM-ACh elicited 200–500 nA of oscillatory current, there was not necessarily a strong suppression of K^+ currents. For these experiments, attenuation was monitored either by clamping oocytes close to the Cl^- reversal potential (−20 to −25 mV), or after injecting oocytes with EGTA (see below). These comparative studies therefore suggested that generation of inositol phosphates and DG in the oocyte itself did not correlate with the potency with which ACh suppressed K^+ responses.

As an alternative approach we looked at the action of serum on K^+ currents and compared this with the attenuation seen with ACh. Rabbit serum elicits large oscillatory Cl^- currents in the oocyte itself through activation of the inositol phosphates–DG pathways (G. Tigyi, C. M. Matute, D. L. Dyer & R. Miledi, unpublished results). For these experiments we selected oocytes with good sensitivity to serum but only small transient Cl^- currents elicited by ACh. Serum was used at dilutions of 1:20000 to 1:40000, which caused little 'non-specific' plugging of membrane currents and, in the same oocyte, was compared with 1–10 μM-ACh for attenuation effects. Although serum elicited oscillatory Cl^- responses, it caused little or no suppression of the K^+ currents. In contrast, ACh elicited much smaller and more transient Cl^- responses, but nevertheless invariably caused 50–70% attenuation (five oocytes, two frogs) (e.g. Fig. 8).

Divalent cations, though eliciting large oscillatory Cl^- responses, were also largely inactive in terms of attenuation. For these experiments the effects of ACh and Co^{2+} were compared only on subsequent K^+ responses (see Van Renterghem *et al.* 1985; Stinnakre & Van Renterghem, 1986). This approach was necessary because divalent cations have 'direct' blocking effects on the cyclic nucleotide-activated K^+ channels.

Finally, we checked whether oocyte Ca^{2+} was necessary for attenuation of K^+ currents. We reasoned that if a messenger pathway involving diacylglycerol and activation of protein kinase C mediated attenuation, then it might be possible to at least partially inhibit the effect by loading oocytes with EGTA. Chelation of oocyte Ca^{2+} was monitored by abolition of native transient outward (T_{out}) currents, which are activated by depolarization (e.g. −80 to +20 mV), are carried by Cl^- and result

from an underlying opening of voltage-gated Ca^{2+} channels in the oocyte membrane (e.g. Miledi, 1982; Leonard, Nargeot, Snutch, Davidson & Lester, 1987). In most of these experiments T_{out} currents were amplified (*ca* 10-fold) by bathing the oocyte in Ringer solution containing 10 mM-Ca^{2+}.

Oocytes were loaded with between 100 and 1500 pmol EGTA, resulting in an intracellular concentration of at least 0·1–1·5 mM. Following injections of 500 pmol EGTA, T_{out} currents were reduced to half in *ca* 60 s and were completely abolished within 5 min. Oocytes were then left for 20–60 min in normal Ringer solution allowing EGTA to diffuse throughout the oocyte and ACh was then tested for its ability to attenuate K^+ responses elicited by forskolin. Injections of EGTA had no clear effect on the level of attenuation developed by ACh (eleven oocytes, four frogs). For example, we compared attenuation in four oocytes injected with 200–600 pmol EGTA with five non-injected oocytes from the same frog. In all cases, 10 μM-ACh suppressed K^+ responses by between 90 and 100% (e.g. Fig. 9). Bathing EGTA-injected oocytes in zero Ca^{2+} Ringer solution also had little effect on the level of attenuation by ACh, though these oocytes tended to gradually lose input resistance.

DISCUSSION

In this paper we describe the membrane current responses elicited by prostaglandins, ANF and oxytocin, and also experiments relating to the mechanism by which ACh attenuates these currents. These are discussed separately below.

Membrane currents elicited by prostaglandins, ANF and oxytocin

Firstly, our experiments show that prostaglandins interact specifically with *Xenopus* ovarian follicles to elicit a membrane current carried substantially by K^+. The potency sequence for this response is:

$$PGE_1 \approx PGE_2 > PGA_1 \geqslant PGA_2 > PGB_1,$$

with $PGF_{2\alpha}$, PGD_2 and PGI_2 having little or no activity. Such selectivity is most simply explained in terms of a single prostaglandin receptor which is sensitive to PGEs. This receptor can be activated to a lesser degree by the related PGAs, the analogue 11-deoxy-PGE_1 and possibly by high concentrations of PGB_1 and 11-β-PGE_2. In contrast, the receptor is largely or wholly insensitive to $PGF_{2\alpha}$, PGD_2, PGI_2 and 8-iso-PGE_1 (cf. Robertson, 1986). Oxytocin and, following pre-incubation with phosphodiesterase inhibitors, also human ANF elicit similar K^+ conductances. Though the pharmacology of these responses remains incomplete our experiments suggest there are specific receptors for PGE, oxytocin and ANF in *Xenopus* follicles. We suspect oxytocin is acting on the same receptor as arginine-vasopressin (see Landau, Moriarty, Gillo & Sealfon, 1987).

Cyclic nucleotides appear to act as intracellular messengers in the receptor–channel coupling mechanism of all these responses. Comparison with various mammalian tissues suggests that the PGE and oxytocin receptors are positively coupled to adenylate cyclase (e.g. Kolena & Channing, 1972; Goff & Armstrong, 1983; Jard, 1983; Robertson, 1986). Because K^+ currents are elicited by intraoocyte injections of either cyclic AMP or cyclic GMP (e.g. Dascal *et al.* 1987; Miledi & Woodward,

1989), it remains unclear whether the *Xenopus* ANF receptor is coupled to guanylate cyclase, as in mammalian ovary (Budnik *et al.* 1987; Pandey *et al.* 1987), or to the follicle's adenylate cyclase.

Prostaglandins, oxytocin and ANF activate a K^+-gating mechanism in common with the follicle's responses to catecholamines, adenosine, gonadotrophins and VIP. Defolliculation experiments suggest that these currents arise in follicular cells which are electrically coupled to the oocyte by gap junctions (e.g. Smith *et al.* 1987; Woodward & Miledi, 1987*a*; Miledi & Woodward, 1989).

There are at least ten distinct receptors for hormones/neurotransmitters all of which elicit the same K^+ response in follicle-enclosed *Xenopus* oocytes: noradrenaline (β-adrenergic), dopamine (D1-dopaminergic), adenosine (P1(Ra)-purinergic), serotonin (pharmacology uncertain), gonadotrophin, prostaglandin (PGE), VIP, ANF, oxytocin–vasopressin and corticotrophin releasing factor (for references see above). Of these, only gonadotrophins have a well defined physiological activity in *Xenopus* (e.g. Masui, 1967; Mulner & Ozon, 1981; Woodward & Miledi, 1987*a*). Experiments described in this paper were prompted by comparing receptor systems present in *Xenopus* follicles with those in the mammalian ovary. Finding PGE and ANF receptors in *Xenopus* reinforces the analogy between receptors which elicit K^+ responses in *Xenopus* follicles (i.e. gonadotrophin, β-adrenergic, VIP, PGE, ANF) and those coupled to cyclic nucleotide synthesis in granulosa cells, in turn suggesting a physiological relevance in *Xenopus* for this plethora of different receptors. In this case, further comparisons with the mammalian ovary could give indications of their physiological activities in *Xenopus* (e.g. Gilula, Epstein & Beers, 1978; Behrman, 1979; Armstrong, 1981; Mulner & Ozon, 1981; Hsueh *et al.* 1983; Eppig & Downs, 1984; Kasson, Meidan, Davoren & Hsueh, 1985; Pandey *et al.* 1987). Alternatively, many of the *Xenopus* receptors might simply be redundant, and control of receptor expression in follicular cells might be compromised by gap junctional contact with the developing oocyte.

The physiological role in *Xenopus* of cyclic nucleotide-activated K^+ currents also remains unclear. Experiments using pFSH, noradrenaline and forskolin show that this membrane current response only becomes pronounced in follicles enclosing oocytes at stages V and VI (Woodward & Miledi, 1987*a*; authors' unpublished results). Considering the apparent similarities between receptor systems found in mammalian and *Xenopus* ovaries it might be interesting to see if cyclic nucleotide-activated K^+ currents are also present in granulosa cells or in the mammalian cumulus cell–oocyte complex.

To conclude, these experiments show that PGE, ANF and oxytocin all elicit K^+ currents in follicle-enclosed *Xenopus* oocytes. These currents appear to be the same cyclic nucleotide-activated K^+ conductance elicited by catecholamines, adenosine, gonadotrophins and VIP. The *Xenopus* ovarian follicle should be a useful system to further investigate the pharmacologies, mechanisms and physiological actions of these receptors.

Attenuation of K^+ responses by ACh

As described, in follicles from many frogs the oocyte membrane itself contains muscarinic receptors coupled to the enzyme phosphoinositidase (phospholipase C),

which stimulate hydrolysis of phosphatidylinositol 4,5-bisphosphate, generating inositol phosphates and DG, both of which act as intracellular messengers (e.g. Oron *et al.* 1985; Parker & Miledi, 1986; for a general review see Berridge, 1987). Activation of this pathway by ACh is monitored electrically due to mobilization of intraoocyte Ca^{2+} by inositol phosphates and activation of Ca^{2+}-gated Cl^- channels in the oocyte membrane (e.g. Miledi, 1982; Oron *et al.* 1985; Parker & Miledi, 1986). In addition, the suppression of cyclic nucleotide-activated K^+ currents by ACh is mimicked by bath-applied phorbol esters, suggesting that this element of ACh responses is mediated by the DG–C-kinase arm of the pathway (Dascal *et al.* 1985). We therefore reasoned that any agonist which stimulated hydrolysis of inositol phospholipids in the oocyte should, through generation of DG, likewise cause attenuation of K^+ responses. Surprisingly, our experiments showed that activation of the pathway by either serum or divalent cations caused little, if any, attenuation of cyclic nucleotide-activated K^+ currents. Similar results have also been reported using follicle-enclosed oocytes injected with brain messenger RNAs, which have induced sensitivity to serotonin (Greenfield, Williams, Vandenberg & Hackett, 1986; authors' unpublished results). Attenuation of cyclic nucleotide-activated K^+ currents therefore appears to be a specific property of the native muscarinic response, and is not mimicked by other agonists which stimulate hydrolysis of inositol phospholipids in the oocyte itself. Our failure to appreciably block attenuation by loading oocytes with high levels of EGTA also indicates that there is no obvious synergism between intraoocyte Ca^{2+} levels and the mechanism by which ACh attenuates K^+ currents.

Results of defolliculation experiments suggest that cyclic nucleotide-activated K^+ responses arise in the follicular layer and unequivocally confirm that muscarinic receptors coupled to inositol phospholipid break-down are located in the oocyte itself (see accompanying paper, Miledi & Woodward, 1989). This raises the problem of how K^+ currents arising in follicular cells are attenuated by an ACh response occurring in the oocyte, presuming the same muscarinic receptors mediate both effects. One possibility is that ACh acts not on the K^+ conductance itself, but merely by reducing the level of electrical coupling between oocyte and follicular cells. Indeed, this type of effect is not unprecedented, as ACh has been shown to regulate the coupling between pairs of rat lacrimal cells (e.g. Neyton & Trautmann, 1986). However, we think this explanation is unlikely. Cyclic nucleotide-activated K^+ responses are not the only membrane currents removed by defolliculation. For example, follicle-enclosed oocytes occasionally have substantial (100–250 nA) voltage-gated K^+ currents, which activate at *ca* -40 mV, and which inactivate only partially over a period of 10 s. In some cases these currents are effectively abolished by manual defolliculation but are nevertheless largely unaffected by exposures to ACh (10–100 μM), which in the same oocytes reduced cyclic nucleotide-activated K^+ currents by 60–80% (authors' unpublished results). If sensitivity to defolliculation is used as a criterion for localizing currents to the follicular cells, then an effect by ACh on the level of coupling would necessarily appear to reduce all currents abolished by defolliculation. The selective insensitivity to ACh of these voltage-gated K^+ currents implies that modulation of coupling is not the major mechanism for attenuation.

In conclusion, the simplest explanation for these experiments is that muscarinic responses arising in the oocyte itself play little or no role in attenuation of cyclic

nucleotide-activated K^+ currents. We therefore propose that the suppression of K^+ responses is mediated by muscarinic receptors located in follicular cells. As previously suggested, the mechanism of attenuation is probably through activation of protein kinase C (Dascal *et al.* 1985), though it remains possible that other mechanisms are involved (e.g. Pfaffinger, Leibowitz, Subers, Nathanson, Almers & Hille, 1988).

This work was supported by a grant from the US Public Health Service (R01-NS 23284). R. M. W was supported in part by the Science and Engineering Research Council of the UK. We are grateful to Sir Bernard Katz for helpful suggestions.

REFERENCES

ADASHI, E. & HSUEH, A. J. W. (1981). Stimulation of β-adrenergic responsiveness by follicle-stimulating hormone in rat granulosa cells *in vitro* and *in vivo*. *Endocrinology* **108**, 2170–2178.

ARMSTRONG, D. T. (1981). Prostaglandins and follicular functions. *Journal of Reproduction and Fertility* **62**, 283–291.

BEHRMAN, H. R. (1979). Prostaglandins in hypothalamo-pituitary and ovarian function. *Annual Review of Physiology* **41**, 685–700.

BERRIDGE, M. J. (1987). Inositol trisphosphate and diacylglycerol: two interacting second messengers. *Annual Review of Biochemistry* **56**, 159–193.

BROWNE, C. L. & WERNER, W. (1984). Intracellular junctions between the follicle cells and oocytes of *Xenopus laevis*. *Journal of Experimental Zoology* **230**, 131–135.

BROWNE, C. L., WILEY, H. S. & DUMONT, J. N. (1979). Oocyte–follicle cell gap junctions in *Xenopus laevis* and the effects of gonadotrophin on their permeability. *Science* **203**, 182–183.

BUDNIK, L. T., BRUNSWIG, B. & MUKHOPADHYAY, A. K. (1987). Atrial natriuretic factor stimulates luteal guanylate cyclase. *Regulatory Peptides* **19**, 23–34.

DASCAL, N., LOTAN, I., GILLO, B., LESTER, H. A. & LASS, Y. (1985). Acetylcholine and phorbol esters inhibit potassium currents evoked by adenosine and cAMP in *Xenopus* oocytes. *Proceedings of the National Academy of Sciences of the USA* **82**, 6001–6005.

DASCAL, N., LOTAN, I. & LASS, Y. (1987). Dissociation of acetylcholine and cyclic-GMP induced currents in *Xenopus* oocytes. *Pflügers Archiv* **409**, 521–527.

DAVOREN, J. B. & HSUEH, A. J. W. (1985). Vasoactive intestinal peptide: a novel stimulator of steroidogenesis by cultured rat granulosa cells. *Biology of Reproduction* **33**, 37–52.

DUMONT, J. N. (1972). Oogenesis in *Xenopus laevis* (Daudin). I. Stages of oocyte development in laboratory maintained animals. *Journal of Morphology* **136**, 153–180.

EPPIG, J. J. & DOWNS, S. M. (1984). Chemical signals that regulate mammalian oocyte maturation. *Biology of Reproduction* **30**, 1–11.

ESPEY, L. L., NORRIS, C. & SAPHIRE, D. (1986). Effect of time and dose of indomethacin on follicular prostaglandins and ovulation in the rabbit. *Endocrinology* **119**, 746–754.

GILULA, N. B., EPSTEIN, M. L. & BEERS, W. H. (1978). Cell-to-cell communication and ovulation. A study of the cumulus–oocyte complex. *Journal of Cell Biology* **78**, 58–75.

GOFF, A. K. & ARMSTRONG, D. T. (1983). Changes in responsiveness of rat granulosa cells to prostaglandin E_2 and follicle-stimulating hormone during culture. *Canadian Journal of Physiology and Pharmacology* **61**, 608–613.

GREENFIELD, L. J., WILLIAMS, E. H., VANDENBERG, S. R. & HACKETT, J. T. (1986). Serotonin receptors induced by brain mRNA in *Xenopus* oocytes are pharmacologically distinct from muscarinic and dopamine receptors. *Society for Neuroscience Abstracts* **119**, 12.

HSUEH, A. J. W., JONES, P. B. C., ADASHI, E. Y., WANG, C., ZHUANG, L. Z. & WELSH, T. H. (1983). Intraovarian mechanisms in the hormonal control of granulosa cell differentiation in rats. *Journal of Reproduction and Fertility* **69**, 325–342.

JARD, S. (1983). Vasopressin: mechanisms of receptor activation. *Progress in Brain Research* **60**, 383–394.

KASSON, B. G., MEIDAN, R., DAVOREN, J. B. & HSUEH, A. J. W. (1985). Identification of subpopulations of rat granulosa cells: sedimentation properties and hormonal responsiveness. *Endocrinology* **117**, 1027–1034.

KLIACHKO, S. & ZOR, U. (1981). Increase in catecholamine-stimulated cyclic AMP and progesterone synthesis in rat granulosa cells during culture. *Molecular and Cellular Endocrinology* **23**, 23–32.

KOLENA, J. & CHANNING, C. P. (1972). Stimulatory effects of LH, FSH and prostaglandins upon cyclic 3′,5′-AMP levels in porcine granulosa cells. *Endocrinology* **90**, 1543–1550.

KUSANO, K., MILEDI, R. & STINNAKRE, J. (1977). Acetylcholine receptors in the oocyte membrane. *Nature* **270**, 739–741.

KUSANO, K., MILEDI, R. & STINNAKRE, J. (1982). Cholinergic and catecholaminergic receptors in the *Xenopus* oocyte membrane. *Journal of Physiology* **328**, 143–170.

LANDAU, E. M., MORIARTY, T. M., GILLO, B. & SEALFON, S. (1987). Endogenous peptide receptors in *Xenopus* oocytes. *Society for Neuroscience Abstracts* **402**, 11.

LEONARD, J. P., NARGEOT, J., SNUTCH, T. P., DAVIDSON, N. & LESTER, H. A. (1987). Ca channels induced in *Xenopus* oocytes by rat brain mRNA. *Journal of Neuroscience* **7**, 875–881.

LOTAN, I., DASCAL, N., COHEN, S. & LASS, Y. (1982). Adenosine induced slow ionic currents in *Xenopus* oocyte. *Nature* **298**, 572–574.

LOTAN, I., DASCAL, N., ORON, Y., COHEN, S. & LASS, Y. (1985). Adenosine induced potassium current in *Xenopus* oocytes and the role of adenosine 3′,5′-mono-phosphate. *Molecular Pharmacology* **28**, 170–177.

MASUI, Y. (1967). Relative roles of the pituitary, follicle cells, and progesterone in the induction of oocyte maturation in *Rana pipiens*. *Journal of Experimental Zoology* **116**, 365–376.

MILEDI, R. (1982). A calcium-dependent transient outward current in *Xenopus laevis* oocytes. *Proceedings of the Royal Society* B **215**, 491–497.

MILEDI, R. & PARKER, I. (1984). Chloride current induced by injection of calcium into *Xenopus* oocytes. *Journal of Physiology* **357**, 173–183.

MILEDI, R., PARKER, I. & WOODWARD, R. M. (1988). Extracellular manganese and cobalt ions activate an oscillatory chloride current in *Xenopus* oocytes. *Journal of Physiology* **400**, 33*P*.

MILEDI, R., PARKER, I. & WOODWARD, R. M. (1989). Membrane currents elicited by divalent cations in *Xenopus* oocytes. *Journal of Physiology* **417**, 173–195.

MILEDI, R. & WOODWARD, R. M. (1989). Effects of defolliculation on membrane current responses of *Xenopus* oocytes. *Journal of Physiology* **416**, 601–621.

MULNER, O. & OZON, R. (1981). The roles of the follicular envelopes in the initiation of *Xenopus* oocyte maturation. *General and Comparative Endocrinology* **44**, 335–343.

NEYTON, J. & TRAUTMANN, A. (1986). Acetylcholine modulation of the conductance of intercellular junctions between rat lacrimal cells. *Journal of Physiology* **377**, 283–295.

ORON, Y., DASCAL, N., NADLER, E. & LUPU, M. (1985). Inositol 1,4,5-trisphosphate mimics muscarinic response in *Xenopus* oocytes. *Nature* **313**, 141–143.

PANDEY, K. N., OSTEEN, K. G. & INAGAMI, T. (1987). Specific receptor mediated stimulation of progesterone secretion and cGMP accumulation by rat atrial natriuretic factor in cultured human granulosa–lutein cells. *Endocrinology* **121**, 1195–1197.

PARKER, I. & MILEDI, R. (1986). Changes in intracellular calcium and in membrane currents evoked by injection of inositol trisphosphate into *Xenopus* oocytes. *Proceedings of the Royal Society* B **228**, 307–315.

PFAFFINGER, P. J., LEIBOWITZ, M. D., SUBERS, E. M., NATHANSON, N. M., ALMERS, W. & HILLE, B. (1988). Agonists that suppress M-current elicit phosphoinositide turnover and Ca^{2+} transients, but these events do not explain M-current suppression. *Neuron* **1**, 477–484.

ROBERTSON, R. P. (1986). Characterization and regulation of prostaglandin and leukotriene receptors: an overview. *Prostaglandins* **31**, 395–411.

SMITH, A. A., BROOKER, T. & BROOKER, G. (1987). Expression of rat mRNA coding for hormone-stimulated adenylate cyclase in *Xenopus* oocytes. *Federation Proceedings* **1**, 380–387.

STINNAKRE, J. & VAN RENTERGHEM, C. (1986). Cyclic adenosine monophosphate, calcium, acetylcholine and the current induced by adenosine in the *Xenopus* oocyte. *Journal of Physiology* **374**, 551–569.

VAN DEN HOEF, N. H. F., DICTUS, W. J. A., HAGE, W. J. & BLUEMINK, J. G. (1984). The ultrastructural organization of gap junctions between follicle cells and the oocyte in *Xenopus laevis*. *European Journal of Cell Biology* **33**, 242–247.

VAN RENTERGHEM, C., PENIT-SORIA, J. & STINNAKRE, J. (1985). β-adrenergic induced potassium current in *Xenopus* oocytes: role of cyclic-AMP, inhibition by muscarinic agents. *Proceedings of the Royal Society* B **223**, 389–402.

WOODWARD, R. M. & MILEDI, R. (1987*a*). Hormonal activation of membrane currents in follicle enclosed *Xenopus* oocytes. *Proceedings of the National Academy of Sciences of the USA* **84**, 4135–4139.

WOODWARD, R. M. & MILEDI, R. (1987*b*). Membrane currents elicited by porcine vasoactive intestinal peptide (VIP) in follicle *Xenopus* oocytes. *Proceedings of the Royal Society* B **231**, 489–497.