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CYCLIC ADENOSINE MONOPHOSPHATE, CALCIUM, ACETYLCHOLINE AND THE CURRENT INDUCED BY ADENOSINE IN THE *XENOPUS* OOCYTE

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SUMMARY

1. The K^+ current response to bath-applied adenosine has been studied on follicle-enclosed full grown oocytes from *Xenopus laevis*, using the two electrodes voltage-clamp technique.

2. The response to adenosine was mimicked by forskolin, an activator of adenylate cyclase.

3. Forskolin applied at low concentration potentiated the response to adenosine.

4. At low concentration, isoprenaline, a β-adrenergic agonist known to induce a potassium current via a rise of adenosine 3′,5′-phosphate (cyclic AMP) into the oocyte, potentiated the response to adenosine.

5. Progesterone (10^{-5} M) reversibly induced a slight decrease (−24%) of the response to adenosine.

6. The calcium ionophore A23187 applied in normal external medium reduced the response to adenosine (about −70%). Intracellular injection of EGTA induced an increase (+64%) of the peak response to adenosine.

7. Acetylcholine (0·5–10 μM) inhibited the response to 3–10 μM adenosine by 44–91%. This inhibition was suppressed by atropine and was seen even on cells which did not show any current in response to acetylcholine application.

8. The inhibition by ACh of the sensitivity to adenosine was long lasting (more than 1 h after the wash-out of ACh). A long term inhibition (−28 to −90%) also occurred when ACh was applied alone and washed before adenosine application.

9. It is concluded that in *Xenopus* oocyte: (i) increased cyclic AMP synthesis mediates the potassium response to adenosine; (ii) intracellular calcium ion concentration modulates this response; (iii) muscarinic stimulation induces a long-lasting inhibition of the sensitivity to adenosine.

INTRODUCTION

Besides their essential role in the energy metabolism of all cells, it is now clear that adenosine and adenosine nucleotides are involved in receptor-controlled regulations of both neuronal and non-neuronal cells (see reviews by Burnstock, 1981; Phillis & Wu, 1981; Stone, 1981; Phillis & Barraco, 1985). Although classification of purinergic

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receptors is still under discussion (see: Brown & Burnstock, 1981; Snyder, 1981; Stone, 1984; Hamprecht & Van Calker, 1985), two classes of purinergic receptors are known to interact with adenylate cyclase.

The *Xenopus* oocyte responds to catecholamines by an increase in K^+ conductance (Kusano, Miledi & Stinnakre, 1977, 1982) which is mediated by a rise in intracellular adenosine 3′,5′-phosphate (cyclic AMP; Van Renterghem, Pénit-Soria & Stinnakre, 1984, 1985). Since adenosine, as well as ATP, ADP or AMP, is able to activate purinergic receptors located on the oocyte membrane (Lotan, Dascal, Cohen & Lass, 1982) we questioned the possibility that the same intracellular messenger could be involved in the potassium current induced by adenosine in this cell.

Using electrophysiological techniques, we show that this is indeed the case and that the sensitivity to adenosine depends on the intracellular free calcium. We also describe a long-lasting inhibition of the response of the oocyte to adenosine by muscarinic agonists.

METHODS

Methods used in the present series of experiments were very similar to those reported by Van Renterghem *et al.* (1985).

Animals and dissection of oocytes

Adult females of *Xenopus laevis* were purchased from Xenopus Ltd., Nutfield, (U.K.), Herpetolisches Institut, Den Dolder, (The Netherland); Snake Farm, Fish Hoek, (South Africa) and Serea, Argenton l'Eglise, (France). No differences were noted in the electrophysiological properties in oocytes from any of the different origins. Animals were kept in tanks filled with continuously flowing tap water maintained between 19 and 22 °C by heating or cooling the reservoirs where chlorine was allowed to escape. The females were fed with calf liver, or beef heart or with granulated trout food, and kept in a 12 h day–night cycle. Adult females were anaesthetized in ethyl *m*-aminobenzoate (MS222) 2 g/l in tap water, an aperture of approximately 1 cm was made in the abdomen and a small piece of ovary was removed. The body wall was stitched up and the animals were left for several weeks until they were used again. Fully grown *Xenopus* oocytes (> 1 mm in diameter; Stages V, VI; Dumont, 1972) were freed from the ovary by manual dissection without any enzymatic treatment. The oocytes obtained in this way were thus still covered by the inner ovarian epithelial layer and the underlying follicular cells and vitelline layer still intact over the oolemma; as such they should be refered to as follicles, however we will use the more common word oocyte throughout this paper for convenience. The oocytes were kept at 12–14 °C in a modified Barth's medium (in mM: $CaCl_2$, 0·45; $Ca(NO_3)_2$, 0·4; KCl, 1; KH_2PO_4, 0·29; $MgSO_4$, 0·84; $NaHCO_3$, 2·37; NaCl, 88; Na_2HPO_4, 0·21; pH 7·4; Barth & Barth, 1959) supplemented with gentamicin (0·1 mg/ml), nystatin (50 u./ml) (cf. Miledi & Sumikawa, 1982) and with fetal calf serum (1 ml/l). In these conditions, the oocytes have been used for up to 10 days following isolation.

In order to expose most of its surface, one oocyte was deposited on a nylon mesh placed horizontally across a chamber continuously perfused at 2·5 ml/min with OR_2 saline (Wallace, Jared, Dumont & Sega, 1973) using a peristaltic pump (Gilson, Minipuls 4). Beside inorganic chemicals (in mM: NaCl, 82·5; KCl, 2·5; $CaCl_2$, 1; $MgCl_2$, 1; Na_2HPO_4, 1; HEPES, 5; pH 7·4;) OR_2 contained glucose (5 mM) and pyruvate (3·6 mM). The saline was admitted in the chamber from below and the volume surrounding the oocytes was kept to about 0·4 ml by a superficial suction. The time necessary for complete concentration change in the chamber was 75 s. The temperature of the solutions was kept at between 20 and 22 °C.

Electrical recordings

Oocytes membrane potential was measured differentially between an intracellular pipette filled with 3 M-KCl (resistance, R, 1–2 MΩ, when measured in OR_2) and an extracellular OR_2-filled pipette ($R < 0·5$ MΩ). The membrane potential was controlled with a voltage-clamp amplifier built

in the laboratory with the high voltage output stage fed through a simple low pass filter (time constant: 0·01/0·1 s). The current-passing pipette was also filled with 3 M-KCl (R = 0·7–1 MΩ). The clamp current was measured with a current to voltage converter the output of which was low pass filtered (time constant: = 0·01 s) and recorded on an ink pen recorder (Brush 280) with faster response than the currents generated by the oocytes. The data shown in the Figures are raw data.

Due to the rather large size of the pipettes necessary for adequate voltage clamp, and to the presence of the inner ovarian epithelium, the penetration of the oocytes was difficult. As a result the initial membrane potential was often low (usually −40 mV). Oocytes were discarded if their membrane potential did not reach −40 mV and their input resistance was less than 0·5 MΩ after 15 min. Most of the oocytes used in the present experiments had a membrane potential between −50 and −70 mV and an input resistance between 0·7 and 1·2 MΩ.

Drugs

The following drugs were purchased from Sigma: acetylcholine (ACh), adenosine, 3′,5′-phosphate (cyclic AMP), atropine, 8-bromoadenosine 3′,5′-phosphate (8-Br-cyclic AMP), ethyleneglycol-bis-(β-aminoethylether)N,N'-tetraacetic acid (EGTA), ethyl m-aminobenzoate (MS 222, tricaine methane sulphonate), Fast Green FCF, (−)-isoprenaline (bitartrate), progesterone, propranolol. Forskolin and the ionophore A23187 were purchased from Calbiochem, methylisobutylxanthine (MIX) from Aldrich Europ and Sigma.

Drug solutions were applied by bath perfusion after the pH was adjusted to 7·4. No solution was recirculated except those containing 8-Br-cyclic AMP. Stock solutions of forskolin were made in ethanol (20 mM) and kept at −18 °C for up to 2–3 months after which they were discarded because it was noticed that their activity was substantially reduced. A 2×10^{-2} M stock solution of A23187 was made in dimethyl sulphoxide (DMSO); the working solution was obtained by diluting the latter slowly while stirring vigorously. Respective final concentrations of A23187 and DMSO were: 1–5 μM/0·7–3·5 mM.

RESULTS

As observed by Lotan *et al.* (1982), an oocyte responded to bath-applied adenosine by an increase in conductance which generated an outward current when the oocyte was clamped at −50 mV. This current was not maintained and decreased slowly to a small value even in the continuous presence of the drug (Fig. 1*A*). When the same dose of adenosine was applied several times in close succession on the same oocyte, the peak response decreased.

The desensitization of the purinergic response was far less pronounced than that observed with isoprenaline (Van Renterghem *et al.* 1985) or with acetylcholine (ACh, Kusano *et al.* 1982; Dascal, Landau & Lass, 1984). In this series of experiments every adenosine application (2 min) was followed by a 12 to 18 min rinse with OR_2. This protocol gave very reproducible responses (see for example Fig. 5) after the first two or three applications which frequently showed some variability. Contrary to Lotan *et al.* (1982) and Dascal *et al.* (1984), we never observed any 'D' (fast inward) current evoked by adenosine. The discrepancy may be due to the lower perfusion rates and concentrations we used.

Forskolin and 8-Br-cyclic AMP both mimic the response to adenosine

Forskolin is known to be a direct activator of the membrane-bound adenylate cyclase in several cell types (Seamon, Padgett & Daly, 1981). In the oocyte, it induces a dose-dependent outward current which reverses direction at the K^+ equilibrium potential (Van Renterghem *et al.* 1985). The same report showed that the cell

permeant and phosphodiesterase-resistant 8-bromo derivative of cyclic AMP was also able to induce an increase in K^+ conductance when applied in the bath.

Fig. 1 provides a comparison of the time course of responses to bath-applied adenosine (5×10^{-6} M), forskolin (10^{-5} M) and 8-Br-cyclic AMP (8×10^{-4} M) on the same cell. The latencies of the responses to adenosine and to forskolin were shorter

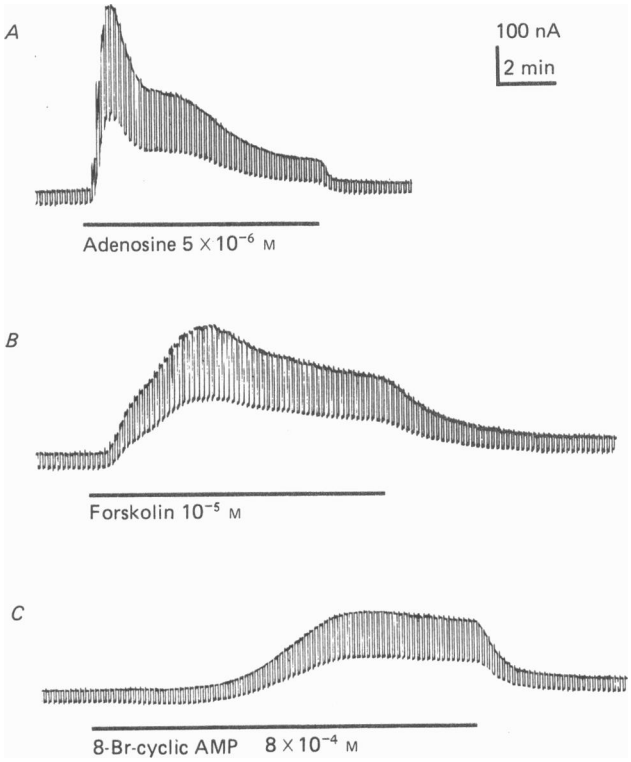

Fig. 1. Comparison of the current responses of the *Xenopus laevis* oocyte to bath-applied adenosine (*A*), forskolin (*B*) and 8-Br-cyclic AMP (*C*). The three records were obtained by voltage clamping the same oocyte alternatively at −50 (upper envelope) and at −70 mV (lower envelope); thus the difference between the two envelopes is an indication of the change in membrane conductance. Capacitive currents masked for clarity. In this Figure as in the others, the dead time of the perfusion system has been subtracted and the bars below the traces correspond to the arrival and to the beginning of the wash-out of the test solutions. Traces are presented in the order they have been recorded.

(respectively approximately 15 and 30 s, Fig. 1*A* and *B*) than that of 8-Br-cyclic AMP: (approximately 4 min, Fig. 1*C*). When a higher concentration of 8-Br-cyclic AMP was used, the latency of the response became shorter than this latter value but remained longer than that of the response to adenosine. The current induced by adenosine rapidly decreased to a low value upon wash-out (about 1 min). The current induced by 8-Br-cyclic AMP disappeared slightly slower (approximately 3 min). On the contrary the current due to forskolin was recorded for a longer period of time after wash-out: 6 min in Fig. 1, but occasionally it remained high for up to 15 min (see for example Fig. 1 of Van Renterghem *et al.* 1985).

Fig. 1 also shows that upon continuous application, the current induced by 8-Br-cyclic AMP decreased very little while that due to forskolin showed a clear decrease. The desensitization of the adenosine current was stronger and developed along two phases.

Forskolin potentiates the adenosine current

At low concentrations which are unable to elicit a potassium current, forskolin strongly potentiates the oocyte response to isoprenaline (Van Renterghem *et al.* 1985). Forskolin when applied on oocytes together with adenosine also caused strong

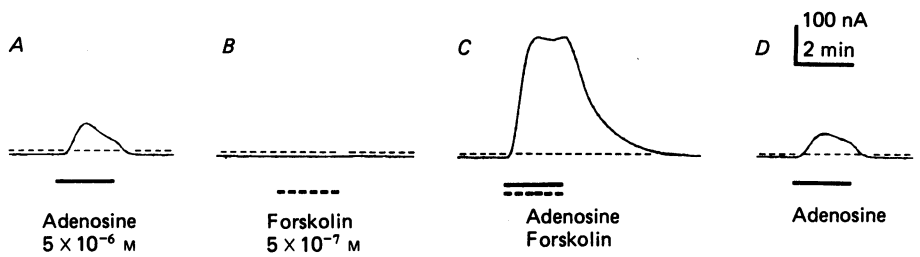

Fig. 2. Potentiation of the adenosine current (*A*) by simultaneous application of 0·5 μM-forskolin (*C*) which did not induce any detectable current when applied alone (*B*). A control adenosine application (*D*) obtained 18 min after *C* gave less current than in *A*. As in the next Figures, the dashed line across the current traces represents the zero current level. Membrane potential was held at −50 mV.

potentiation (Fig. 2). While forskolin at 0·5 μM did not induce any current (Fig. 2*B*), when applied in combination with adenosine (5 μM) an increase of the adenosine current of 280% was produced (Fig. 2*C*) which was reversible (Fig. 2*D*). In five applications on three oocytes from two females the average potentiation factor was 2·82 (S.D. = 0·85). It can be observed also that in the presence of forskolin, the adenosine current is better maintained than in control conditions. Also, it was normally observed that the response to a control adenosine application following a test in the presence of forskolin was slightly smaller than the response obtained before forskolin (average ratio 0·45; S.D. = 0·22; $n = 4$).

Effects of phosphodiesterase inhibitors

Inhibitors of phosphodiesterases might be expected to enhance the response to adenosine as they do on the response to β-adrenergic agonists (Van Renterghem *et al.* 1985). Xanthine derivatives which are commonly applied as phosphodiesterase blocking agents cannot be easily used here for this purpose because they also act as antagonists at the purinergic receptors (Snyder, 1981; Lotan *et al.* 1982; Van Renterghem *et al.* 1985).

However some potentiating effect of methylisobutylxanthine (MIX) on the adenosine response could be demonstrated: when an application of adenosine was washed with OR_2 containing MIX instead of normal OR_2, a transient increase of the K^+ current was recorded before the current trace returned to the base line (Fig. 3*A*). If MIX was only blocking the adenosine receptor, one would expect either no change

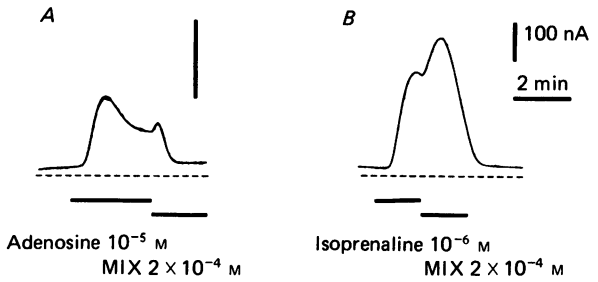

Fig. 3. Transient effect obtained by washing-out adenosine (10 μM, *A*) or isoprenaline (1 μM, *B*) by a saline containing methylisobutylxanthine (MIX, 2×10^{-4} M).

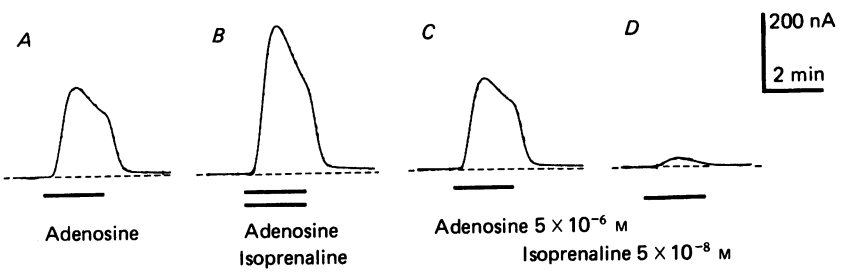

Fig. 4. Potentiation of the adenosine current (*A*) by a simultaneous application of 5×10^{-8} M-isoprenaline (*B*). The same concentration of isoprenaline induced a small current (*D*) when tested 13 min after *C*. This current was slightly greater at the beginning of the experiment (200 min earlier: 40 nA instead of 20 nA). Holding membrane potential: −50 mV.

in the decay rate or a faster decrease if during the slow decrease in bath concentration which occurs during washing some adenosine molecules reassociate with the receptors. The latency for this effect was too short to be estimated in our conditions of perfusion. A similar phenomenon could be observed when a β-adrenergic agonist was replaced by MIX in the perfusion fluid (Fig. 3*B*, same cell). As a rule however, this procedure induced a larger increase after an isoprenaline application (45 % relative to the actual current value) than after an adenosine one (13 %, two applications each).

Effect of a β-adrenergic agonist

It is known that the *Xenopus* follicle contains β-adrenergic receptors (Kusano *et al.* 1982) whose activation leads to an increase in intracellular cyclic AMP concentration (Van Renterghem *et al.* 1985). It is thus expected that a β-adrenergic agonist like isoprenaline should enhance the response to adenosine.

'Cross potentiation' was tested using isoprenaline at concentrations close to its half-maximal effective concentration (Van Renterghem *et al.* 1984) in combination with two concentrations of adenosine. As illustrated in Fig. 4, adenosine (5×10^{-6} M) and isoprenaline (5×10^{-8} M) induced respectively currents of 225 and 20 nA. When the two substances were applied simultaneously the current reached 370 nA showing a potentiation of 51 % relative to the sum of the two responses. Reduction of the

isoprenaline concentration (3×10^{-8} M) produced a smaller potentiation (+33%) but potentiation was larger if a smaller concentration of adenosine was used. In the presence of the above concentrations of isoprenaline ($3–5 \times 10^{-8}$ M), adenosine-(2×10^{-6} M) induced responses respectively 83 and 50% larger than the sum of the control responses.

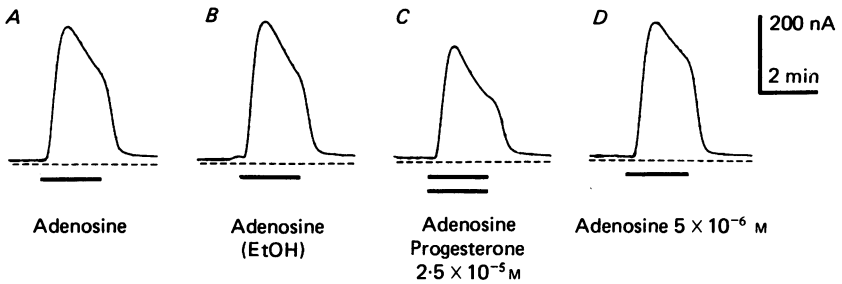

Fig. 5. Effect of progesterone (25 μM) applied simultaneously for 2 min together with adenosine (*C*). Control adenosine (5 μM) applications performed in normal OR_2 (*A*) and in the presence of an equal amount of ethanol (*B*) are also shown. The reduction of the adenosine response by progesterone is no longer observed on the next control (15 min later, *D*). Interval between tests: 17 min (*A–B*), 21 min (*B–C*). Holding potential −50 mV.

On the contrary, higher doses of isoprenaline produced a reduction of the adenosine current: the effect of 0·1 μM of isoprenaline (inducing a change in membrane conductance of up to 20 μS) on a *subsequent* application of adenosine was tested on three oocytes from three donors. Isoprenaline produced a 36% mean decrease of the response to adenosine applied 3 min after washing-out the isoprenaline.

This inhibition could have been obtained if adenosine were in fact acting through the β-adrenergic receptor previously desensitized by the isoprenaline application. That was not the case because propranolol (10^{-6} M) did not change the current induced by adenosine (10^{-5} M). However, propranolol (10^{-5} M) did reduce slightly (13%, mean of five experiments) the current due to adenosine (50–100 μM).

Effect of progesterone

Progesterone is the natural signal which triggers the maturation process in *Xenopus* oocyte (Smith, 1975). It has been shown to lower the basal concentration of cyclic AMP in *Xenopus* oocytes (Maller, Butcher & Krebs, 1979), to decrease the synthesis of cyclic AMP by the oocyte (Mulner, Huchon, Thibier & Ozon, 1979) and to inhibit the adenylate cyclase activity of plasmic membrane isolated from defolliculated oocytes after treatment with cholera toxin or guanosine 5′-(β,γ-imido)triphosphate (Gpp(NH)p, Finidori-Lepicard, Schorderet-Slatkine, Hanoune & Baulieu, 1981; Jordana, Allende & Allende, 1981; Sadler & Maller, 1981). It was thus of interest to know if progesterone was capable of blocking the current induced by adenosine.

Bellé, Ozon & Stinnakre (1977) reported that progesterone (10^{-6} M) did not produce any significant change either in membrane potential or in membrane resistance during the 2–4 h following progesterone application. In accordance with this report no

current change was observed when progesterone (10^{-6}–$2 \cdot 10^{-5}$ M) was applied for 2 or 3 min on an oocyte (three observations on three cells). When progesterone was applied together with adenosine (5×10^{-6} M) the response to the latter decreased by 24% relatively to controls performed in the presence of the same concentration of ethanol as in the test (mean of two oocytes). This effect was quickly reversible (Fig. 5) and could be reproduced on the same cell.

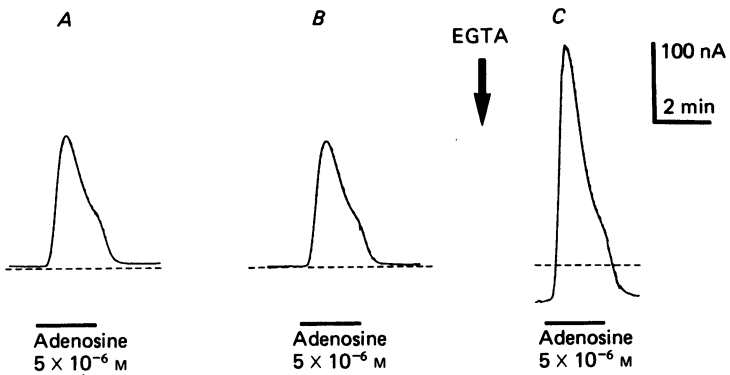

Fig. 6. Effect of intracellular injection of a solution containing 0·2 M-EGTA and Fast Green (*C*) on the response to adenosine (*A*, *B*). 40 min elapsed between control application *B* and test *C* during which the EGTA pipette was inserted into the oocyte producing a drop in membrane resistance seen as an increased holding current. An adenosine application (not shown) was made between *B* and *C* to keep the test interval constant. No attempt was made to measure the injected volume. Holding potential: −50 mV.

Effects of manipulating intracellular calcium concentration

In their study of the action of isoprenaline on the oocyte, Van Renterghem *et al.* (1985) noticed that the calcium-ionophore A23187 almost completely suppressed the β-catecholamine response. On the other hand, calcium ions are known to interfere with oocyte phosphodiesterases via a calcium–calmodulin complex (Echeverria, Orellana, Jedlicki, Plaza, Allende & Allende, 1981; Miot & Erneux, 1982). It was then likely that adenosine-induced current was sensitive to intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$). This was tested by either injection of the calcium chelator EGTA or treatment of the preparation with the ionophore A23187.

EGTA (0·2 M, pH adjusted to 7·4 with KOH) was pressure injected (Stinnakre, 1979) together with Fast Green (concentration not measured) as to follow injection progress (but two 'blind' injections without the dye proved to be as efficient). To prevent unwanted leakage of EGTA into the cell during control experiments, the EGTA containing pipette was inserted into the oocyte just prior to injection, with the consequence of increasing the holding current. No control injections without EGTA were performed.

Fig. 6 shows that injection of EGTA into an oocyte increased the adenosine current. After EGTA injection, performed in four oocytes (from three females), the current induced by adenosine 5×10^{-6} M increased by 10–129% (mean 61%, S.E. of mean = 23·8). Two other 'injections' in which Fast Green was included had no effect

on the adenosine current, however it is probable that no chelator was injected because contrary to the previous experiments no green colour could be observed in the oocyte.

To increase the resting $[Ca^{2+}]_i$ the ionophore A23187 which is known to carry preferentially divalent cations across membranes (Pfeiffer & Lardy, 1976) was used.

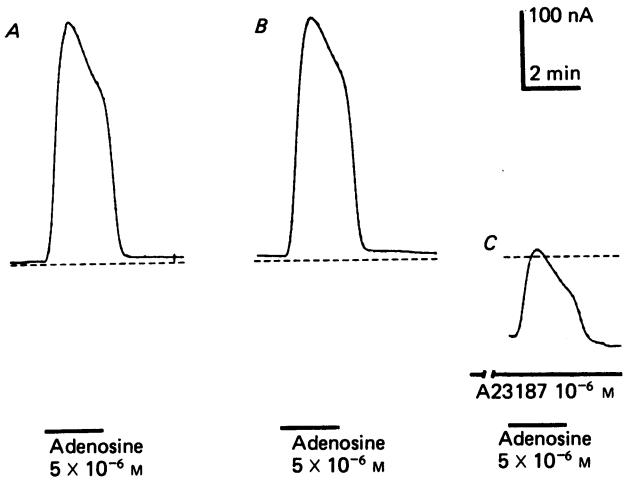

Fig. 7. Reduction of the adenosine response by pre-treatment of the oocyte with 2 μM-A23187 and 1·4 mM-DMSO (*C*). No DMSO was present in control *A*, *B*. Interval between adenosine applications: 22 min; holding potential: −48 mV.

Pre-incubation of five oocytes with A23187/DMSO produced an increase of membrane conductance of 1–5 μS. This increase was apparently not related to the concentrations used (1–5 μM) as if they were already giving a maximal effect. The corresponding current which was inward at −50 mV reversed at −24 mV (mean of four oocytes), a value close to E_{Cl} (Kusano *et al.* 1982). Presumably it represented the calcium-activated chloride current described by Miledi (1982), Barish (1983) and Miledi & Parker (1984).

The current elicited by 5 μM-adenosine applications was measured after a pre-incubation period of at least 4 min with the ionophore. In two oocytes from two donors, the amplitude of the outward current was reduced to 35 and 27 % relative to control responses obtained in the absence of both A23187 and DMSO (Fig. 7). These values may be compared to those obtained by Van Renterghem *et al.* (1985) in their study of isoprenaline and 8-Br-cyclic AMP. As shown on Table 1, where the conductance induced by the pre-incubation with A23187 is also indicated, it appears that the inhibition of the adenosine-induced K^+ current correlated very well with the conductance induced by the ionophore itself. Thus this conductance may be an indication of the actual $[Ca^{2+}]_i$. The possible effect of DMSO alone on the adenosine responses was not tested in this series of experiments, however it has been observed that DMSO did not modify the potassium response induced by isoprenaline (C. Van Renterghem, unpublished observation).

TABLE 1. Comparison of the changes in conductance and of the drug inhibition induced by treatment with A23187

A23187 (μM)	Δ Conductance (S)	Drug	Concentration (M)	Inhibition (%)
1	$5{\cdot}4 \times 10^{-6}$	Isoprenaline	5×10^{-8}	87
5	$7{\cdot}8 \times 10^{-6}$	Isoprenaline	5×10^{-8}	100
1	$5{\cdot}4 \times 10^{-6}$	8-Br-cyclic AMP	10^{-3}	73
1	$4{\cdot}7 \times 10^{-6}$	Adenosine	5×10^{-6}	65
5	$5{\cdot}0 \times 10^{-6}$	Adenosine	5×10^{-6}	73

ACh depresses the adenosine-induced current

The effect of ACh on the oocyte adenosine response was tested using several concentrations of either adenosine or ACh (3–10 μM for the former, 0·5–10 μM for the latter), the oocytes being clamped at either −50 mV or at −10 mV, a value close to the chloride equilibrium potential (cf. Kusano *et al.* 1982). Test and control adenosine applications were applied at regular intervals (14–20 min, see Methods), ACh being either mixed with one adenosine solution or applied 4–8 min before an adenosine application (see Fig. 8). In all cases ACh was applied for 2 min.

Inhibition of the adenosine current was quite variable but it was observed in all oocytes tested whether an ACh (Cl^- dependent) current was recorded or not (Fig. 8*A–C*). In sixteen oocytes from six animals, ACh depressed the adenosine current by at least 31 % when applied simultaneously with adenosine (mean = 59 %, $n = 10$, S.E. of mean = 6·7, not corrected for the eventual ACh-induced current) and by at least 28 % (mean = 60 %, $n = 10$, S.E. of mean = 7·5) when applied alone. In one more case, the depression was only 9 %. In three oocytes, the ACh inhibition could be observed two or three times in succession after sufficient time had elapsed to allow for recovery (see below).

As has been described for isoprenaline (Van Renterghem *et al.* 1985) the depressing effect of ACh on the adenosine current was blocked by a pre-treatment with atropine indicating that the inhibition was obtained by activation of muscarinic receptors.

Due to the rather large variability of the inhibition it has not yet been possible to know whether the clamp potential could influence the process. We do not know either if inhibition depends on the duration of ACh application on the oocyte membrane.

The ACh inhibition is long lasting

As noticed previously the inhibitory effect of ACh could still be observed 4–8 min after ACh was applied alone, it therefore over-lasted the presence of ACh itself. In seven oocytes out of eight which received ACh and adenosine simultaneously, the subsequent adenosine response was below control while all eight which received ACh alone still showed a clear inhibition at the second test. When the inhibition of the successive adenosine responses was plotted *versus* time, a linear relationship was observed on a semi-logarithmic scale (Fig. 9). Time constant for recovery varied from 12 to 36 min for simultaneous application and from 18 to 56 min in the other case; however, we do not have enough experiments to test for a significant difference. We

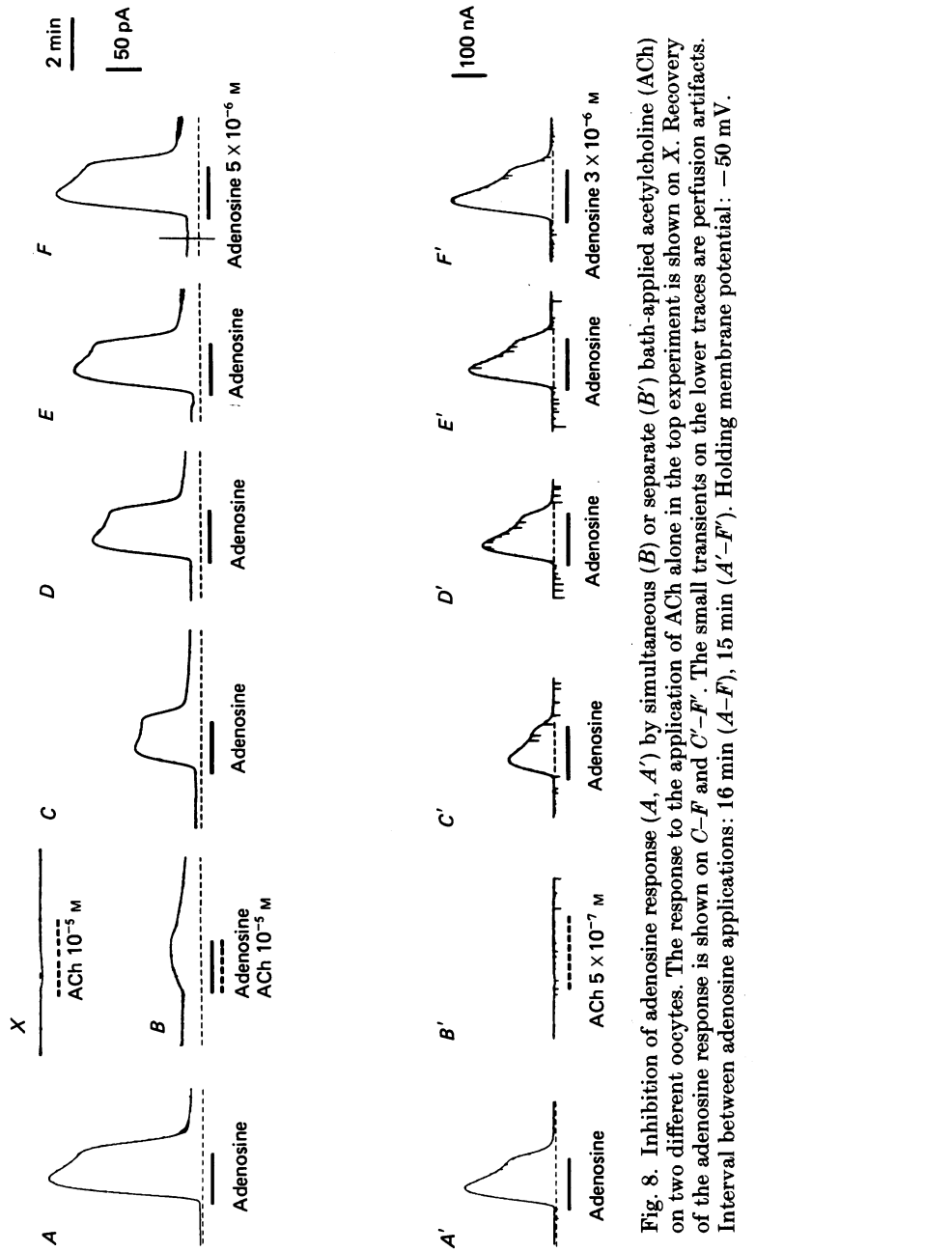

Fig. 8. Inhibition of adenosine response (*A*, *A'*) by simultaneous (*B*) or separate (*B'*) bath-applied acetylcholine (ACh) on two different oocytes. The response to the application of ACh alone in the top experiment is shown on *X*. Recovery of the adenosine response is shown on *C*–*F* and *C'*–*F'*. The small transients on the lower traces are perfusion artifacts. Interval between adenosine applications: 16 min (*A*–*F*), 15 min (*A'*–*F'*). Holding membrane potential: −50 mV.

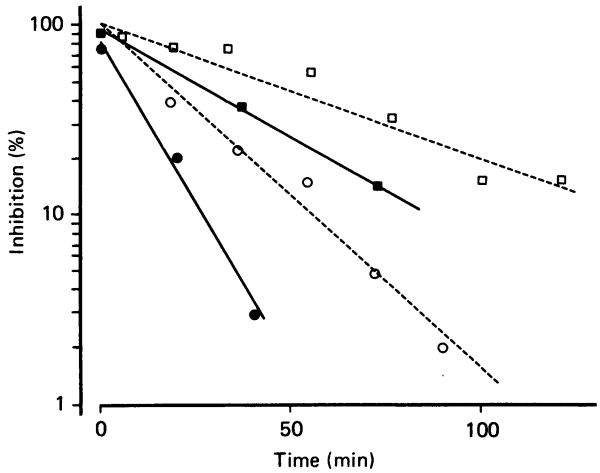

Fig. 9. Semi-logarithmic plots of the inhibition of the adenosine response after simultaneous (filled symbols) or separate ACh application (open symbols). Inhibition was computed as:

$$(I_{control} - I_{test}) \times 100 / I_{control},$$

where $I_{control}$ and I_{test} are the adenosine-induced K^+ currents respectively measured before and during or after ACh application. In all experiments ACh was applied for 2 min at 10 μM. Adenosine was applied at 5 μM (except for the filled circles: 10 μM) every 18 min (filled square, open circles), 20 min (filled circles) and 14–24 min (open squares). Holding potential was −50 mV except for the filled circles (−14 mV). Lines fitted by eye.

are still unable to demonstrate whether a correlation exists between ACh inhibition of the adenosine response and ACh-induced current when it exists.

DISCUSSION

Cyclic AMP is the intracellular messenger of the adenosine-induced potassium current

Adenosine perfusion of the *Xenopus* oocyte induces a K^+ conductance increase (Lotan *et al.* 1982). Although all the steps leading from the receptor activation to the opening of the K^+ channels have not been identified, there is little doubt that adenosine acts by increasing the intracellular cyclic AMP concentration. Indeed, it is mimicked by forskolin, an activator of adenylate cyclase (Seamon *et al.* 1981) which is active on the adenylate cyclase of the *Xenopus* oocyte membrane (Jordana, Olate, Allende & Allende, 1984), by 8-Br-cyclic AMP, a cyclic AMP analogue (Meyer & Miller, 1974) and by isoprenaline (Kusano *et al.* 1982).

As shown for isoprenaline (Van Renterghem *et al.* 1984, 1985), forskolin, at doses which do not induce any current, is also capable of very much enhancing the sensitivity of the oocyte to adenosine. This effect probably corresponds to the fact that cyclic AMP-synthesis stimulation by agonists of receptors positively coupled to adenylate cyclase is increased in the presence of forskolin in other systems (Seamon *et al.* 1981). Isoprenaline which has been shown to act via a rise in intracellular cyclic AMP concentration (Van Renterghem *et al.* 1984, 1985) also potentiates the adenosine current, and it seems that if these two latter substances were not acting through a common pathway one would not observe such potentiations, i.e. a more than additive action.

On the other hand, progesterone, the natural inducer of maturation (Smith, 1976) which inhibits the adenylate cyclase activity of the oocyte membrane (Maller *et al.* 1979; Mulner *et al.* 1979; Finidori-Lepicard *et al.*, 1981: Jordana *et al.* 1981), reduced the adenosine response.

We do not have clear data showing that inhibition of the phosphodiesterases, the enzymes which normally degrade cyclic nucleotides, also augments the oocyte response to adenosine. Indeed xanthines which are commonly used to block these enzymes are also known to inhibit purinergic receptors (Snyder, 1981; Lotan *et al.* 1982; Van Renterghem *et al.* 1985) and therefore the oocyte cannot be pre-treated with this class of inhibitors. However, the transient increase in adenosine current observed when interrupting the adenosine (or the isoprenaline) perfusion with MIX (or a non-xanthine inhibitor, C. Van Renterghem unpublished observation) is in agreement with a phosphodiesterase inhibition. It suggests that these enzymes are easily accessed from the outside of the cell and they may be linked to the membrane. Indeed a membrane-associated phosphodiesterase activity has been reported by Echeverria *et al.* (1981, see also Miot & Erneux, 1982). Papaverine could have been used instead of MIX but as it did not give clear results in preliminary experiments it was not used further. From the quick effect of MIX it can be assumed on the other hand that the latencies of the isoprenaline and the adenosine responses come from the time necessary for cyclic AMP accumulation rather than from the subsequent events leading to the opening of the K^+ channels.

The slight difference in latencies between the adenosine and the forskolin current is consistent with the site of action of the latter being located on the plasma membrane and that the activation of the adenylate cyclase through the receptor pathway is only a little faster than the time forskolin takes to reach and bind to this enzyme; however, the dissociation of the adenylate cyclase–forskoline complex must take far longer than the normal deactivation of the enzyme when the agonist is removed. The rather faster reversibility of the potentiating effect over the direct action may be however attributed to the lower concentrations used in the first case.

On the contrary, the slower 'on' kinetics of 8-Br-cyclic AMP probably suggests that its site of action is the normal cyclic AMP target, the latency representing the time necessary to build up a sufficiently high concentration. Although 8-Br-cyclic AMP is described as a 'good' *in vitro* activator of the cyclic AMP-activated protein kinase and as resistant to phosphodiesterases (Meyer & Miller, 1974), it is likely that the required concentration be relatively high because cyclic AMP itself is unusually high in *Xenopus* oocytes: about 4 μM (O'Connor & Smith, 1976). On the other hand it is unlikely that 8-Br-cyclic AMP acts in blocking phosphodiesterases and thus elevating endogenous cyclic AMP because we have observed that MIX is nearly unable to evoke a change in resting conductance and biochemical studies have also shown that theophylline and papaverine induce a light increase of the resting intracellular concentration of cyclic AMP (Maller *et al.* 1979) or have no effect (Bravo, Otero, Allende & Allende, 1978). It is worth noting that the wash-out of 8-Br-cyclic AMP is very much quicker than the start of its action.

The observation that the responses to higher concentrations of adenosine were relatively less enhanced than the responses to low concentrations is consistent with observations (C. Van Renterghem, J. Penit-Soria & J. Stinnakre, unpublished observations) that responses to isoprenaline and to forskolin at high doses were less

than additive. A saturation might occur if the number of adenylate cyclase molecules available for activation by the two different receptor systems approaches its maximum value. However, Jordana *et al.* (1984) have reported that maximal stimulation of the oocyte membrane adenylate cyclase activity *in vitro* by Gpp(NH)p and forskolin were additive. Thus, the non-additivity we have observed probably did not result from a property of the adenylate cyclase but from a depletion of the cytoplasmic adenosine triphosphate which is used in cyclic AMP synthesis and in putative cyclic AMP-controlled phosphorylations.

It follows from the above discussion that the purinergic receptor present in the oocyte membrane belongs to the A_2 type as defined by Snyder (1981): the receptor which activates a membrane-bound adenylate cyclase via a GTP-binding regulatory protein. The presence of two different systems for cyclase activation is indeed confirmed by the observation that MIX, the purinergic-receptor inhibitor, has no antagonistic action on the effect of isoprenaline, while propranolol, the β-adrenergic antagonist does not block the adenosine sensitivity. The slight inhibitory effect of propranolol at 10 μM on the adenosine response can be compared to its ability to induce oocyte maturation (Baulieu, Godeau, Schorderet & Schorderet-Slatkine, 1978) and to inhibit cholera toxin-induced cyclic AMP accumulation (Schorderet-Slatkine, Schorderet & Baulieu, 1982) both of which being possibly related to an unspecific increase in intracellular calcium.

Intracellular calcium and response to adenylate cyclase activators

Our data clearly show that a presumed increase of $[Ca^{2+}]_i$ using the ionophore A23187 decreases the purinergic and the β-adrenergic-induced K^+ current while a chelation with EGTA of the intracellular free Ca^{2+} increases it. These results are just opposite to the well known calcium-dependent potassium conductance (Meech, 1972) and to the calcium-dependent chloride conductance more recently described in several preparations including the oocyte (Miledi, 1982; Barish, 1983; Miledi & Parker, 1984). Therefore it is likely that calcium ions do not act at the channel level but rather at some earlier step(s).

Calcium concentration may act on the metabolism of cyclic nucleotides at various locations and, indeed, interactions between $[Ca^{2+}]_i$ and the metabolism of cyclic nucleotides has been described (see review by Rasmussen & Goodman, 1977). A negative action of calcium ions may be obtained if they activate phosphodiesterases. In *Xenopus* oocyte (Echeverria *et al.* 1981; Miot & Erneux, 1982) as in other cells there exist calcium-independent phosphodiesterases and phosphodiesterases activated by a calmodulin–calcium complex. Moreover intracellular injection of calmodulin decreases the cyclic AMP level (Mulner, Tso, Huchon & Ozon, 1983).

Since the inhibiting effect of $[Ca^{2+}]_i$ has been observed also on the 8-Br-cyclic AMP response, it is unlikely that the main action of calcium ions is to inhibit the adenylate cyclase since this enzyme is not directly involved in the effect of 8-Br-cyclic AMP and shows a low basal activity (Finidori-Lepicard *et al.* 1981). Thus calcium ions may interfere with the events leading to the channel opening or again with the phosphodiesterase: perhaps 8-Br-cyclic AMP acts on the potassium conductance together with the basal cyclic AMP concentration and it is only the latter which is reduced by a calcium-activated phospodiesterase. Alternatively 8-Br-cyclic AMP may no longer be resistant to the esterases in the presence of calcium.

The muscarinic inhibition of the adenosine response

The inhibition of the adenosine-induced increase in K^+ conductance by applied ACh closely resembles the effect of ACh on the isoprenaline-induced current (Van Renterghem *et al.* 1985). As both actions turn out to be mediated through an activation of the adenylate cyclase and a subsequent increase in cytoplasmic cyclic AMP, it seems appropriate to assume that in both cases the ACh inhibition is due to the same mechanism.

A blocking action can take place at the adenylate cyclase level or a later stage of the events leading to the potassium current. As discussed in detail by Van Renterghem *et al.* (1985) muscarinic inhibition of β-adrenergic increased accumulation of cyclic AMP has been studied in several cell types. Both activation of phosphodiesterase activity in whole cells and inhibition of adenylate cyclase activity of membranes have been reported. In cardiac cells, Hazeki & Ui (1981) and Harden, Scheer & Smith (1982) have shown that this last effect involves the membrane-linked GTP-binding inhibitory regulatory protein of the membrane-bound adenylate cyclase (so-called N_i or G_i, see Codina, Hildebrand, Iyengar, Birnbaumer, Sekura & Manclark, 1983; and reviews by Rodbell, 1980 and Gilman, 1984).

In the present study it is clear that not only the inhibitory effect of ACh is long lasting, but also ACh does not need to be present at the time of adenosine (and also isoprenaline, not shown) application; therefore the inhibition is not due to the simultaneous action of the agonist and the antagonist on some membrane site. However, it is not clear yet whether ACh may show an inhibition when the K^+ response has started although a preliminary experiment suggests it does. Such a long-lasting inhibitory effect contrasts with that observed in cardiac cells where the muscarinic inhibition of the β-adrenergic enhancement of action potential has been reported to be fully reversible in less than 10 min (Biegon & Pappano, 1980).

The slow kinetics of recovery from muscarinic inhibition shows an exponential characteristic whose time constant is in the order of tens of minutes. The apparent first-order kinetics suggest that muscarinic inhibition is dependent on a single molecular complex whose dissociation is slowly reversible.

It can be argued that the inhibition by either ACh or progesterone might involve a rise in intracellular calcium ions since we have observed that an increase in $[Ca^{2+}]_i$ reduces the adenosine current while a decrease does the opposite. Indeed, an increase in intracellular free calcium has been observed following progesterone treatment of non-pigmented *Xenopus* oocyte (Wasserman, Pinto, O'Connor & Smith, 1980) and it has been reported that some of the ACh-induced currents in the oocyte require the external presence of calcium ions and disappear in the presence of the calcium inhibitor verapamil (Dascal & Landau, 1980; Dascal *et al.* 1984). Also ACh was shown recently to increase the production of inositol tri-phosphate ($InsP_3$) which can induce a chloride current when microinjected in oocytes (Oron, Dascal, Nadler & Lupu, 1985). It has been suggested that muscarinic receptors lead to an intracellular release of calcium ions triggered by $InsP_3$ (Michell, 1975; see Berridge & Irvine, 1984 for review). Thus activation of a cyclic nucleotide phosphodiesterase (or an inhibition of cyclic AMP effects) might contribute to the decrease of the potassium response to adenosine.

However, in the case of progesterone, an inhibition of the adenylate cyclase activity

has been reported to occur in a membrane fraction (Finidori-Lepicard *et al.* 1981) and does not seem to require a calcium step. A GTP-binding protein is involved (Jordana *et al.* 1981; Sadler & Maller, 1981). But contrary to other cyclase inhibitors, progesterone does not seem to involve the N_i complex since its effect is not blocked by pertussis toxin (Katada & Ui, 1981; Olate, Allende, Allende, Sekura & Birnbaumer, 1984). Undoubtedly, this intramembrane interaction plays a role in the inhibitory action of progesterone we report here.

In the case of ACh, it is unlikely that a long-lasting rise in $[Ca^{2+}]_i$ could be responsible for the inhibition of the adenosine response. Indeed, it is known that a rise in the intracellular calcium activity of the oocyte induces a calcium-dependent chloride permeability which we certainly observe during the application of the ionophore A23187. If the ACh inhibition was carried out by a sustained rise in $[Ca^{2+}]_i$ ACh would trigger a long-lasting chloride current which is not observed (Kusano *et al.* 1977, 1982); furthermore inhibition was also observed in cells where no ACh-activated chloride current could be recorded. Although it is possible that the two phenomena might occur at quite different concentrations (the inhibition being obtained at low $[Ca^{2+}]_i$ and the induction of the chloride current at high $[Ca^{2+}]_i$), it seems probable that a rise in cytosolic Ca^{2+}, if major in the inhibitory effect of ACh would be only transitory and then serve as a trigger for some other long-lasting process.

Presently we feel that the inhibition takes places at the membrane level because preliminary experiments seem to indicate that (i) 8-Br-cyclic AMP response is not reduced by simultaneously applied ACh (while it is reduced by treatment with A23187), (ii) the response to forskolin is only weakly sensitive to ACh. This inhibition would then be of the same nature as that of the interaction between muscarinic and β-adrenergic agonists studied in heart cells where it involves the N_i unit. The long-lasting character which is reported here can be expected to exist in other systems. It is likely that stimulation of breakdown of phosphatidylinositol (Oron *et al.* 1985) and inhibition of production of cyclic AMP do occur together in the same cell.

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REFERENCES

BARISH, M. E. (1983). A transient calcium-dependent chloride current in the immature *Xenopus* oocyte. *Journal of Physiology* **342**, 309–325.

BARTH, L. G. & BARTH, L. J. (1959). Differentiation of cells of the *Rana pipiens* gastrula in unconditioned medium. *Journal of Embryology and Experimental Morphology* **7**, 210–222.

BAULIEU, E.-E., GODEAU, F., SCHORDERET, M. & SCHORDERET-SLATKINE, S. (1978). Steroid-induced meiotic division in *Xenopus laevis* oocytes: surface and calcium. *Nature* **275**, 593–598.

BELLÉ, R., OZON, R. & STINNAKRE, J. (1977). Free calcium in full grown *Xenopus laevis* oocytes following treatment with ionophore A23187 or progesterone. *Molecular and Cellular Endocrinology* **8**, 65–72.

BERRIDGE, M. J. & IRVINE, R. F. (1984). Inositol triphosphate, a novel second messenger in cellular signal transduction. *Nature* **312**, 315–321.

BIEGON, R. L. & PAPPANO, A. J. (1980). Dual mechanism for inhibition of calcium dependent action potentials by acetylcholine in avian ventricular muscle. *Circulation Research* **46**, 353–362.

BRAVO, R., OTERO, C,. ALLENDE, C. C. & ALLENDE, J. E. (1978). Amphibian oocyte maturation and protein synthesis: related inhibition by cyclic AMP, theophylline and papaverine. *Proceedings of the National Academy of Sciences of the U.S.A.* **75**, 1242–1246.

BROWN, C. M. & BURNSTOCK, G. (1981). Evidence in support of the P_1/P_2 purinoceptor hypothesis in the guinea-pig taenia coli. *British Journal of Pharmacology* **73**, 617–624.

BURNSTOCK, G. (1981). Neurotransmitters and trophic factors in the autonomic nervous system. *Journal of Physiology* **313**, 1–35.

CODINA, J., HILDEBRAND, J., IYENGAR, R., BIRNBAUMER, L., SEKURA, R. & MANCLARK, C. (1983). Pertussis toxin substrate, the putative N_i component of adenylyl cyclases is a 2β heterodimer regulated by guanine nucleotide and magnesium. *Proceedings of the National Academy of Sciences of the U.S.A.* **80**, 4276–4280.

DASCAL, N. & LANDAU, E. M. (1980). Types of muscarinic response in *Xenopus* oocytes. *Life Sciences* **27**, 1423–1428.

DASCAL, N., LANDAU, E. M. & LASS, Y. (1984). *Xenopus* oocyte resting potential, muscarinic responses and the role of calcium and guanosine 3′,5′-cyclic phosphate. *Journal of Physiology* **352**, 551–574.

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

ECHEVERRIA, M., ORELLANA, O., JEDLICKI, E., PLAZA, M., ALLENDE, C., & ALLENDE, J. E. (1981). Regulation of a cyclic nucleotide phosphodiesterase from *Xenopus laevis* ovary by calmodulin and calcium. *Biochemistry International* **2**, 539–546.

FINIDORI-LEPICARD, J., SCHORDERET-SLATKINE, S., HANOUNE, J. & BAULIEU, E. E. (1981). Steroid hormone as regulatory agent of adenylate cyclase. Inhibition by progesterone of the membrane bound enzyme in *Xenopus laevis* oocytes. *Nature* **292**, 255–257.

GILMAN, A. G. (1984). G proteins and dual control of adenylate cyclase. *Cell* **36**, 577–579.

HAMPRECHT, B. & VAN CALKER, D. (1985). Nomenclature of adenosine receptors. *Trend in Pharmacology* **6**, 153–154.

HARDEN, T. K., SCHEER, A. G. & SMITH, M. H. (1982). Differential modification of the interaction of cardiac muscarinic cholinergic and beta-adrenergic receptors with a guanine nucleotide binding component(s). *Molecular Pharmacology* **21**, 570–580.

HAZEKI, O. & UI, M. (1981). Modification by islet-activating protein of receptor-mediated regulation of cyclic AMP accumulation in isolated rat heart cells. *Journal of Biological Chemistry* **256**, 2856–2862.

JORDANA, X., ALLENDE, C. & ALLENDE, J. (1981). Guanine nucleotides are required for progesterone inhibition of amphibien oocytes adenylate cyclase. *Biochemistry International* **3**, 527–532.

JORDANA, X., OLATE, J., ALLENDE, C. & ALLENDE, J. (1984). Studies on the mechanism of inhibition of amphibian oocyte adenylate cyclase by progesterone. *Archives of Biochemistry and Biophysics* **228**, 379–387.

KATADA, T. & UI, M. (1981). Islet activating protein. A modifier of receptor-mediated regulation of rat islet cyclase. *Journal of Biological Chemistry* **256**, 8310–8317.

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.

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

MALLER, S. M., BUTCHER, F. R. & KREBS, E. G. (1979). Early effect of progesterone on levels of cyclic adenosine 3′,5′-monophosphate in *Xenopus* oocytes. *Journal of Biological Chemistry* **254**, 579–582.

MEECH, R. W. (1972). Intracellular calcium injection causes increased potassium conductance in *Aplysia* nerve cells. *Comparative Biochemistry and Physiology* **42A**, 493–499.

MEYER, R. B. & MILLER, J. P. (1974). Analogs of cyclic AMP and cyclic GMP: general methods of synthesis and the relationship of structure to enzymic activity. *Life Sciences* **14**, 1019–1040.

MICHELL, R. H. (1975). Inositol phospholipids and cell surface receptor function. *Biochimica et biophysica acta* **415**, 81–147.

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. & SUMIKAWA, K. (1982). Synthesis of cat muscle acetylcholine receptors by *Xenopus* oocytes. *Biomedical Research* **3**, 390–399.

MIOT, F. & ERNEUX, C. (1982). Characterization of the soluble cyclic nucleotide phosphodiesterases in *Xenopus laevis* oocytes. Evidence for a calmodulin-dependent enzyme. *Biochimica et biophysica acta* **701**, 253–259.

MULNER, O., HUCHON, D., THIBIER, C. & OZON, R. (1979). Cyclic AMP synthesis in *Xenopus laevis* oocytes. Inhibition by progesterone. *Biochimia et biophysica acta* **582**, 179–184.

MULNER, O., TSO, J., HUCHON, D. & OZON, R. (1983). Calmodulin modulates the cyclic AMP level in *Xenopus* oocyte. *Cell Differentiation* **12**, 211–218.

O'CONNOR, C. M. & SMITH, L. D. (1976). Inhibition of oocyte maturation by theophylline: possible mechanism of action. *Developmental Biology* **52**, 62–65.

OLATE, J., ALLENDE, C. C., ALLENDE, J., SEKURA, R. D. & BIRNBAUMER, L. (1984). Oocyte adenylyl cyclase contains N_i, yet the guanine nucleotide-dependent inhibition by progesterone is not sensitive to pertussis toxin. *Federation of European Biochemical Societies Letters* **175**, 25–30.

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

PFEIFFER, D. R. & LARDY, H. A. (1976). Ionophore A23187: the effect of H^+ concentration on complex formation with divalent and monovalent cations and the demonstration of K^+ transport mediated by A23187. *Biochemistry* **15**, 935–943.

PHILLIS, J. W. & BARRACO, R. A. (1985). Adenosine, adenylate-cyclase, and transmitter release. *Advance in Cyclic Nucleotides Research* **19**, 243–257.

PHILLIS, J. W. & WU, P. H. (1981). The role of adenosine and its nucleotides in central synaptic transmission. *Progress in Neurobiology* **16**, 187–239.

RASMUSSEN, H. & GOODMAN, D. B. (1977). Relationship between calcium and cyclic nucleotides in cell activation. *Physiological Review* **57**, 421–509.

RODBELL, M. (1980). The role of hormone receptors and GTP-regulatory proteins in membrane transduction. *Nature* **284**, 17–22.

SADLER, S. E. & MALLER, J. L. (1981). Progesterone inhibits adenylate cyclase in *Xenopus* oocytes. Action of the guanine nucleotide regulatory protein. *Journal of Biological Chemistry* **256**, 6368–6373.

SCHORDERET-SLATKINE, S., SCHORDERET, M. & BAULIEU, E. E. (1982). Cyclic-AMP mediated control of meiosis. Effect of progesterone, cholera toxin and membrane active drugs in *Xenopus laevis* oocytes. *Proceedings of the National Academy of Sciences of the U.S.A.* **79**, 850–854.

SEAMON, K. B., PADGETT, W. & DALY, D. W. (1981). Forskolin: unique diterpene activator of adenylate cyclase in membranes and in intact cells. *Proceedings of the National Academy of Sciences of the U.S.A.* **78**, 3363–3367.

SMITH, L. D. (1975). Molecular events during oocyte maturation. In *Biochemistry of animal development*, vol. 3, ed. WEBER, R., pp. 1–46. New York, London: Academic Press.

SNYDER, S. H. (1981). Adenosine receptors and the actions of methylxanthines. *Trends in Neurosciences* **40**, 242–244.

STINNAKRE, J. (1979). Pressure injection of aequorin into molluscan neurone. In *Measurement of free calcium in cells*, ed. ASHLEY, C. C. & CAMPBELL, A. K., pp. 189–201. Amsterdam: Elsevier/North Holland.

STONE, T. W. (1981). Physiological roles for adenosine and adenosine 5′ triphosphate in the nervous system. *Neuroscience* **6**, 523–555.

STONE, T. W. (1984). Purine receptors classification. A point for discussion. *Trends in Pharmacological Sciences* **5**, 492–493.

VAN RENTERGHEM, C., PÉNIT-SORIA, J. & STINNAKRE, J. (1984). β-adrenergic induced potassium current in *Xenopus* oocyte: involvement of cyclic AMP. *Biochimie* **66**, 135–138.

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

WASSERMAN, W. J., PINTO, L. H., O'CONNOR, C. M. & SMITH, L. D. (1980). Progesterone induces a rapid increase in $(Ca^{2+})_{in}$ of *Xenopus laevis* oocytes. *Proceedings of the National Academy of Sciences of the U.S.A.* **78**, 1534–1536.

WALLACE, R. A., JARED, D. W., DUMONT, J. N. & SEGA, M. W. (1973). Protein incorporation by isolated amphibian oocytes. III. Optimum incubation conditions. *Journal of Experimental Zoology* **184**, 321–334.