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Adenosine-Induced K^+ Current in *Xenopus* Oocyte and the Role of Adenosine 3′,5′-Monophosphate

ILANA LOTAN, NATHAN DASCAL, YORAM ORON, SASSON COHEN, AND YORAM LASS

The Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv 69978, Israel

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SUMMARY

Voltage clamp technique was used in *Xenopus laevis* oocytes in order to study and compare membrane currents evoked by extracellularly applied adenosine (0.1–10 μM) and intracellularly injected cyclic AMP (0.15–10 μM). The adenosine response is a late long-lasting outward K^+ current ("H" current), mediated by the Ra purine receptor subtype. The H current amplitude is directly proportional to (occupancy)^3; the K_D for adenosine is 3.34 μM. The H current is inhibited by the intracellular injection of protein kinase inhibitors, types II and III (5–450 ng/oocyte) and is usually potentiated by *intracellular* injection of theophylline (100–300 μM), though *extracellular* application of theophylline (1–100 μM) reversibly blocks the receptor. Occasionally, the H current is contaminated by a small Cl^- current. The cyclic AMP current is also a long-lasting K^+ outward current which is potentiated by extracellular theophylline (2 mM). Injection of cyclic AMP inhibits the membrane response to subsequent application of adenosine. The converse inhibition of a cyclic AMP response by an earlier adenosine response is also observed but at very high concentrations of adenosine (>0.6 mM). It was shown by radioimmunoassay that extracellular adenosine increases the level of the intracellular cAMP within a few seconds by about 30%. Intracellular injection of a comparable amount of cAMP was shown to evoke a measurable K^+ current. It is proposed that the adenosine-evoked K^+ outward current is mediated by a rise in intracellular cAMP.

INTRODUCTION

It has been suggested that cyclic nucleotides may mediate slow membrane responses to neurotransmitters (1, 2). Many authors demonstrated that cAMP[1] plays a role in mediating membrane responses to serotonin in molluscan neurons (3–5) and to β-adrenergic agonists in the heart (6–8). Although purines are known to evoke membrane responses in many tissues (9–14) and to modulate adenylate cyclase activity (15), the intermediary role of cAMP in membrane purinergic responses has not yet been directly demonstrated.

In a previous publication (16), we briefly reported the occurrence of purine-evoked membrane currents in *Xenopus* oocytes, of which a K^+ outward current ("H" current) is a major component. The H current is slow and appears after a long delay following the application of adenosine, suggesting a sequence of biochemical events between the adenosine stimulus and the opening of K^+ channels. The H current has been ascribed to the activation of the P1 purine receptors known to *modulate* cAMP levels in other preparations (17). The present work is a more detailed electrophysiological, pharmacological, and biochemical study of this current. We show that the pharmacology of the H current corresponds to that of the Ra purine receptor subtype (18) which is known to *increase* intracellular cAMP levels (19). Also, intracellular injection of cAMP evokes a long-lasting outward current, identical in its time course and its ionic mechanism with the H current. We further present evidence that cAMP may be the intracellular second messenger of the adenosine-evoked H current in *Xenopus* oocytes.

EXPERIMENTAL PROCEDURES

Preparation and Solutions

The experiments were performed on fully grown *Xenopus laevis* oocytes (stages 5 and 6, Ref. 20) from mature female frogs, purchased from the South African Snake Farm (Fish Hoek). The oocytes were used as follicles, i.e., the outer cellular layers were not removed, since it was shown earlier (16) that the ooctye purinergic response originates at the oocyte membrane.

The solutions used in the study are listed in Table 1. The experiments were performed at room temperature (20–24°). The pH of all solutions was 7.4–7.5. All the solutions used in a single experiment had

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[1] The abbreviations used are: cAMP, adenosine 3′,5′-monophosphate; TEA, tetraethylammonium; NECA, 5′-*N*-ethylcarboxamide-adenosine; 5′-AMP, adenosine 5′-monophosphate; IBMX, 3-isobutyl-1-methylxanthine; PKI, protein kinase inhibitor; PDE, phosphodiesterase; L-B, Lineweaver-Burk.

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the same pH (within ±0.02 unit) and the same osmolarity. Except for the ion under study and its substitute, the concentrations of all ions were kept constant. Usually the experiments were performed in normal Ringer's solution. When the effect of $[K^+]_{out}$ was studied, the intermediate concentrations of this ion were obtained by mixing solutions 2 and 3 in different proportions.

Electrophysiology

Since the electrophysiological recording system was described in detail elsewhere (21), only a brief description will be given here. A single oocyte was placed in a 1-ml bath and constantly perfused with one of the solutions listed in Table 1. Changes of the solutions in the bath or bath application of drugs (dissolved in one of the solutions used in the study) were performed without changing the rate of flow of the perfusion system. Purines were applied at 15–30-min intervals to avoid desensitization. The cell was penetrated with two conventional 3 M KCl electrodes and voltage clamped.

The study of the voltage current characteristics. This and the response reversal potential were performed by applying a ramplike change of the holding potential (duration, 0.8 sec). The voltage measurement circuit was connected to the *x* axis of a *X-Y* plotter, the current measurement circuit was connected to the *y* axis, and the voltage current relationship (*V-I* curve) was plotted automatically. The reversal potential of the evoked response was measured from the crossover point of two *V-I* curves, one obtained just before the application of the drug and the other obtained at the plateau of the response (see Ref. 21 and Fig. 2A).

Intracellular injections. These were preformed by insertion of a third microelectrode filled with one of the following solutions: 2 M TEA, 0.1–0.25 M cAMP (sodium salt), 0.02 M 5′-AMP (sodium salt), 0.02 M theophylline, 0.02 M IBMX, 20 mg/ml PKI-type II, 116 mg/ml PKI-type III, or 60 mg/ml bovine serum albumin.

Pressure injections were performed using broken-tip microelectrodes (2–4-μm tip diameter) to which pressure was applied from a compressed gas cylinder. The injected volume was determined before and after the experiment by injecting the drug into paraffin oil (21).

Iontophoretic injections were performed by micropipette which was connected to a constant current unit (Grass CCU1). The iontophorectic current was measured by the voltage clamp circuit (21).

The Determination of cAMP Levels

cAMP levels were determined by radioimmunoassay, essentially as described by Harper and Brooker (22). Briefly, 10 oocytes were incubated in a minimal volume of Ringer's solution (10–20 μl/ooctye) with and without adenosine. The reaction was terminated by homogenization in 0.5–1.0 ml of 5% trichloroacetic acid. The acid was removed by ether extractions, and the extract was diluted and acetylated with acetic anhydride. To avoid possible artifacts of adenosine cross-reacting with the anti-cAMP antibody, oocytes were incubated in very small volumes of normal Ringer's solution (to limit the amount of the added adenosine). Identical quantities of adenosine were also added to control oocytes after the homogenization step (though under these conditions, control cAMP levels remained unchanged whether the adenosine was added or not). Because of the relatively small effects of adenosine on the intracellular concentrations of cAMP we have validated our results by checking multiple dilutions of the extracts, including internal standards in the assayed samples, and performed independent assays with a commercial radioimmunoassay kit on lower dilutions of unacetylated extracts.

Materials

TEA was purchased from B.D.H. Anti-cAMP antibody was a generous gift of Dr. Gary Brooker, University of Virginia School of Medicine. [^{125}I]iodotyrosylmethylsuccinyl-cAMP was either purchased from Sigma or synthesized from tyrosylmethylsuccinyl-cAMP (Sigma) by iodination with chloramine T and purified in our laboratory. [^{125}I] Sodium iodide and radioimmunoassay kits were purchased from Amersham Corp. NECA was a generous gift of Byk Gulden Pharmazeutica.

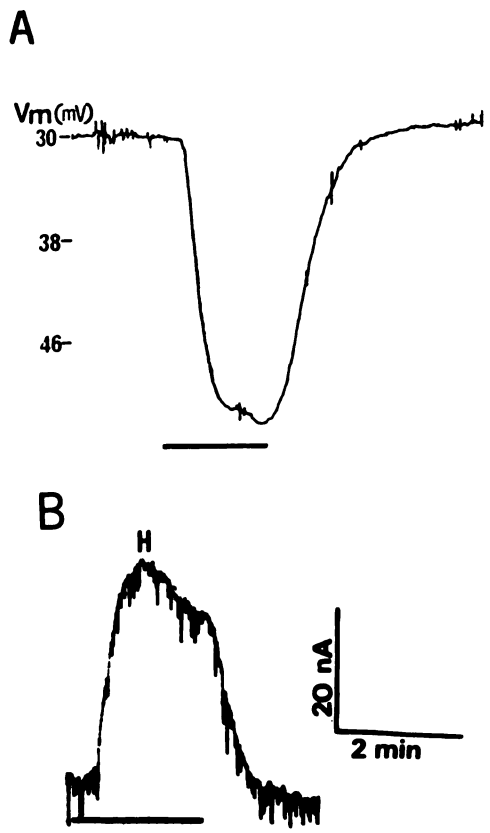

FIG. 1. *A typical response of oocytes to adenosine (H response)* The *horizontal bars* denote the duration of the application. A). In a voltage trace of the response, adenosine (20 μM) elicited a slow hyperpolarization with a long delay. Same time scale as in B. B). In a current trace of the response under voltage clamp conditions, adenosine (0.1 mM) elicited an outward current. The holding potential was −50 mV.

PKI-type II (from beef heart) and -type III (from porcine heart), and all other fine chemicals and drugs were obtained from Sigma, Israel.

RESULTS

The adenosine-induced K^+ outward current (H current). A typical response (H response) elicited by adenosine is shown in Fig. 1. This response was the dominant purinergic response in the oocyte. It was a slow hyperpolarization of the membrane potential (Fig. 1A) produced by a slow outward current (Fig. 1B) with a latency of 25–60 sec well beyond the "dead time" of the perfusion system (5–10 sec).

The H current was blocked by TEA, a K^+ channel blocker (23). TEA was applied either extracellularly (10–40 mM) or even more effectively, intracellularly by an iontophoretic current (see "Experimental Procedures"), with an average amplitude of 200 nA and duration of about 45 min (6 oocytes, 6 frogs). Assuming that the transfer number of TEA is 1 and the oocyte volume is 1 μl, the intracellular concentration of TEA was about 5 mM.

The reversal potential of the H current in a normal Ringer's solution was −107.3 ± 4.36 mV (mean ± S.E., 17 oocytes, 13 frogs). A strict Nernst relationship was obtained between the reversal potential of the H current and the external K^+ concentration, $[K^+]_{out}$; the slope of the plot of the reversal potential against log $[K^+]_{out}$ was

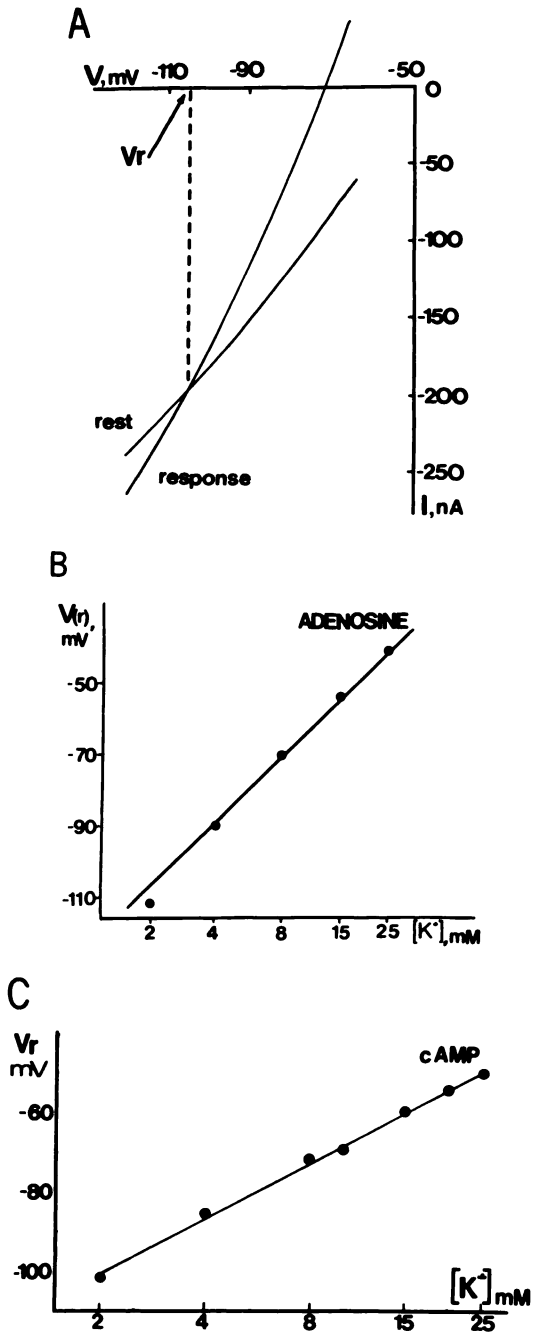

FIG. 2. *Study of voltage current characteristics*

A). *V-I* curves at rest and at the plateau of the response obtained by the "ramp" method (see "Experimental Procedures"). The reversal potential of the response was determined from the crossover point of the two *V-I* curves obtained automatically on the *X-Y* plotter. In this specific case it was a cAMP response in normal Ringer's solution (K^+_{out} = 2 mM); the *V-I* curve at the plateau of the response has a larger slope indicating an increase in membrane conductance during the response. The reversal potential of the response was −102 mV. B). The dependence of the reversal potential of the H response on $[K^+]_{out}$ in a single oocyte. The reversal potential was measured using the "ramp" method. In order to change $[K^+]_{out}$, solutions 2 and 3 (see Table 1) were used. The *straight line* has a slope of 58 mV per a 10-fold change in $[K^+]_{out}$. C). The dependence of the reversal potential, V_r (cAMP), on $[K^+]_{out}$ in a single oocyte. The *straight line* has a slope of 46 mV per a 10-fold change in $[K^+]_{out}$.

58 ± 1 mV (mean ± S.E., 9 oocytes, 9 frogs) per a 10-fold change in $[K^+]_{out}$ (Fig. 2B). It was thus concluded that the H current was carried by K^+ ions. Based on the measured reversal potential and the Nernst equation, the intracellular concentration of K^+ ions was calculated to be 126.2 mM. This value correlates well with the intracellular K^+ activity (a^i_K) in *Xenopus* oocytes, which was measured to be 121.5 mM (24).

Occasionally, in some frogs, the response to adenosine consisted of an inward Cl^- current underlying the H current (16). In such cases the measured reversal potential of the H current was less negative than −100 mV at K^+_{out} = 2 mM. As a consequence, we studied the H current in oocytes in which the reversal potential was measured to be more negative than −100 mV. In an alternative course, the study of the H current was carried out in oocytes being clamped at or near the Cl^- equilibrium potential that was estimated from the reversal potential of the acetylcholine-induced fluctuations which are carried by Cl^- ions (21, 24). This reversal potential was usually between −20 and −30 mV.

We had shown before that the H current could be reversibly blocked by externally applied theophylline (1–100 μM) and was mediated by P1 purine receptors (16). In this paper we further characterized the receptor subtype as Ra (18), on the basis of the potency sequence of agonists: NECA > adenosine > N^6-phenylisopropyladenosine (Fig. 3).

The H current, when elicited either by NECA or by adenosine, was dose dependent and saturable (Fig. 4A). The Hill coefficient was 2.78 ± 0.2 (Mean ± S.D., 5 oocytes, 4 frogs) implying that a cooperative action of at least 3 agonist molecules is involved in generating the H current (25). The data were consistent with a model which assumes that the response is a linear function of (occupancy)^3. "Occupancy" is defined as [agonist]/(K + [agonist]), where [agonist] is the applied concentration and K is the equilibrium dissociation constant of the agonist-receptor complex. This model is expressed as follows.

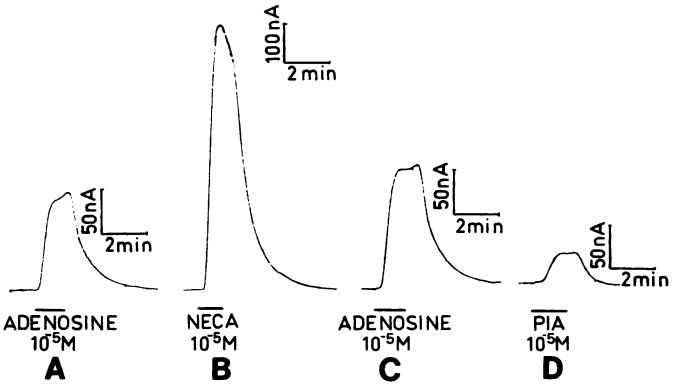

FIG. 3. *Consecutive equimolar applications of various purinergic agonists in the same cell*

The holding potential was −50 mV. Drugs were applied at 15-min intervals. The control H current (A) of adenosine was 110 nA; the current induced by NECA (B) was markedly increased to 595 nA (note reduced calibration); the control current induced by adenosine following NECA (C) was slightly augmented to 135 nA; the current induced by PIA (D) was only 35 nA.

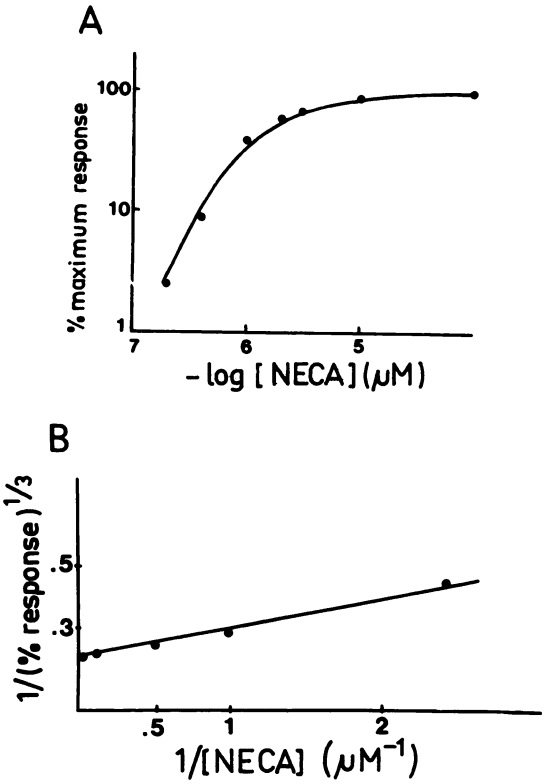

FIG. 4. *The does-response characteristics of the NECA-evoked H response in a single oocyte*

The holding potential was −50 mV. A). A plot of the amplitude of the H response *versus* NECA concentration, the *solid line* was drawn using Equation 1 with K_H = 0.43 nM and H_{max} = 950 nA. These values were obtained from the Lineweaver-Burk plot (B). B). The modified Lineweaver-Burk plot derived from Equation 1. Same experiment as in A; the maximum slope measured between the two initial points of the Hill plot was 2.94 (not shown). The *straight line* was fitted by eye.

$$H_{obs} = H_{max}\left(\frac{[agonist]}{K_H + [agonist]}\right)^3 \quad (1)$$

H_{obs} and H_{max} are the observed and the maximal response amplitudes.

Indeed, a modified Lineweaver-Burk plot (L-B plot) of $H_{(obs)}^{-1/3}$ against $[agonist]^{-1}$, derived from this model, proved to be linear (see Fig. 4B), and substitution of the K_H and H_{max} values obtained from the analysis of the L-B plot provided a satisfactory fit to the experimental data (Fig. 4A, *solid line*).

We considered also the more conventional model which is expressed as follows.

$$H_{obs} = H_{max}\frac{[agonist]^3}{K_H + [agonist]^3} \quad (2)$$

However, in this case a modified L-B plot of $(H_{obs})^{-1}$ against $[agonist]^{-3}$ was not linear, and the fit with Equation 2 was poor.

Thus, the K_H values were calculated from the L-B plot of Equation 1. The K_H for adenosine was found to be 3.34 ± 1.55 μM (mean ± S.D., 5 oocytes, 4 frogs) while the K_H for NECA was 0.3 and 0.15 μM in the two cells studied (2 frogs).

cAMP evoked K^+ current in the oocyte. Typical responses to intracellular iontophoresis of cAMP are shown in Fig. 5. The response was a dose-dependent transient slow outward current with a latency of 30–90 sec and a time-to-peak of 3–6 min. Repeated small cAMP responses (up to 50 nA), at 15-min intervals, were reproducible. However, larger responses induced some inhibition of subsequent cAMP responses that lasted for several hours (see below ("Interaction between the adenosine H and cAMP currents.") cAMP responses, similar to those presented in Fig. 5, were obtained in 15 cells (6 frogs). When cAMP was applied intracellularly by pressure injection (allowing the evaluation of the injected volume, see "Experimental Procedures"), a threshold response (few nA at −50 mV holding potential) could be detected at about 0.15 pmol/oocyte (3 oocytes, 3 frogs) while 5–10 pmol elicited large responses (100–800 nA at −50 mV holding potential). In cells that readily responded to cAMP, intracellular iontophoretic application of 5′-AMP (3 oocytes, 2 frogs) was ineffective, even in very large doses.

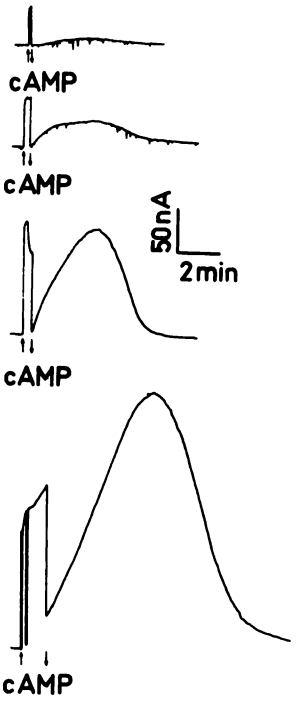

FIG. 5. *Dose-dependent cAMP responses in a single oocyte*

The cell was clamped at −50 mV. A third microelectrode filled with cAMP (0.1 M sodium salt) was inserted into the cell, and cAMP was injected iontophoretically by a negative pulse. The iontophoretic current pulse was measured by the clamp circuit.

Extracellular application of 1 mM IBMX (2 oocytes, 2 frogs) or 2 mM theophylline (3 oocytes, 2 frogs) increased both the duration and the amplitude of the cAMP response (Fig. 6A), indicating that the injected cAMP was degraded by phosphodiesterase in the absence of the inhibitor. Occasionally, phosphodiesterase inhibitors induced a prolonged outward current (see Fig. 6A, 4 oocytes, 4 frogs), presumably due to an increase in basal levels of cAMP.

The cAMP response resulted from an increase in oocyte membrane conductance, as shown by the increased slope of the *V*-I curve at the plateau of the response (Fig.

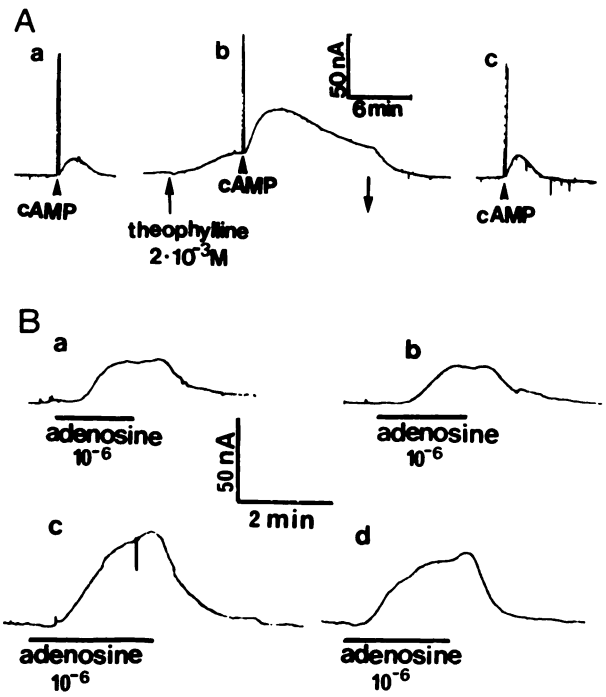

FIG. 6. *Effects of extracellularly applied and injected theophylline*

The effect of extracellularly applied theophylline on the response to injected cAMP. The membrane potential was clamped at −40 mV. a). Control cAMP response. b). Augmentation of the cAMP response superimposed on a theophylline-induced outward current. c). A cAMP response after 1 hr of theophylline washout. B). The effect of injected theophylline on response to extracellularly applied adenosine. The phosphodiesterase inhibitor was injected into the cell. The membrane potential was clamped at −40 mV. a, b). Control responses to adenosine (two samples out of four consecutive identical responses). c). Adenosine response 3 min after pressure injection (see "Experimental Procedures") of 200 pmol of theophylline. d). Adenosine response 100 min after theophylline injection.

TABLE 1

The composition of the solutions used in the study (in mM)

All solutions listed in the table contained 5 mM Tris-HCl and 1.8 mM Ca^{2+}.

Solution	$[Na^+]$	$[K^+]$	$[Mg^{2+}]$	$[Cl^-]$	[Sucrose]
1. Normal Ringer's	116	2	1	128.6	
2. Low K^+	93	0	13.5	128.6	12.5
3. High K^+	93	25	1	128.6	

2A). In normal Ringer's solution the cAMP response reversal potential was −99.05 ± 9.01 mV (mean ± S.D., 10 oocytes, 8 frogs). A plot of the reversal potential against log $[K^+]_{out}$ (Fig. 2C) had a slope of 47.37 ± 1.97 mV (mean ± S.D., 4 oocytes, 3 frogs) for a 10-fold change in $[K^+]_{out}$. It was thus concluded that an increase in membrane K^+ conductance is the underlying mechanism of the cAMP membrane response.

The effect of adenosine on intracellular cAMP level. The basal cAMP level in the oocytes was 1.39 ± 0.15 pmol/oocyte (14 frogs). The changes in cAMP levels were calculated in each experiment (a single frog) relative to the basal level of cAMP in control oocytes obtained from the same frog. The results of all experiments were averaged as shown in Table 1. Adenosine caused a moderate but consistent elevation in intracellular cAMP concentration which was maximal in the first few seconds of exposure (28 ± 6%, mean ± S.E., 5 frogs, see Table 1). Adenosine also increased cAMP levels in denuded oocytes (16).[2] Similar increases in cAMP concentrations produced by adenosine were measured when the assay was validated by multiple dilutions, inclusion of internal standards in the assay samples, or by assaying concentrated unacetylated extracts (see legend to Table 2).

TABLE 2

Adenosine-induced cAMP changes in the Xenopus oocyte

Xenopus oocytes from a frog were incubated in 5–10 μl of normal Ringer's solution in the absence or in the presence of 0.1 mM adenosine. The reaction was terminated by addition of trichloroacetic acid and, usually, the oocytes were extracted and cAMP determined as described under "Experimental Procedures" (12 experiments, 12 frogs). In two additional experiments (2 frogs), the acetylation step was omitted, and the concentrated extracts were assayed with a commercial kit. The basal level of intracellular cAMP determined in unstimulated oocytes was 1.39 ± 0.15 pmol/oocyte (14 experiments, 14 frogs).

Time of exposure to adenosine	Increase in cAMP concentration[a]
sec	%
3–5	28 ± 6 (5), $p < 0.005$
10	27 ± 11 (4), $p < 0.05$
30	22 ± 9 (5), $p < 0.05$
90–120	11 ± 5 (14), $p < 0.025$

[a] The values are mean ± S.E. (number of experiments in different frogs). Probability values were calculated using paired *t* test.

The effect of intracellularly injected phosphodiesterase inhibitors on the H current. One of the accepted methods to demonstrate a link between changes in intracellular cAMP and agonist-induced biological responses is to employ PDE inhibitors which potentiate the response. However, in the case of adenosine, PDE inhibitors block the adenosine receptor at concentrations lower by 1–2 orders of magnitude than those required for the inhibition of PDE (26). We tried to prevent the exposure of adenosine receptors to PDE inhibitors by intracellular pressure injection of theophylline (100–300 pmol/oocyte). Indeed, the amplitude of the H response to adenosine was agumented by 86.2 ± 38.6% (mean ± S.D., 4 oocytes, 4 frogs, the range was 40–125%) with respect to control responses in the same oocyte before the injection of PDE inhibitor. This potentiation was not reversible for as long as 2 h after the injection (Fig. 6B). However, in two additional cells injected with 100 pmol of IBMX and 700 pmol of theophylline, inhibition rather than potentiation of the H current was observed, presumably due to outward diffusion of theophylline or IBMX across the membrane and eventual blockade of the purinergic receptors.

Protein kinase inhibitors suppress the adenosine H current. It is generally accepted that cAMP exerts its intracellular effects through the activation of cAMP-dependent protein kinase. We have tested the effect of intracellularly injected protein kinase inhibitors (PKI, types II and III) on the adenosine response. PKI-II (5–

[2] Y. Oron and S. Gelerstein, unpublished results.

450 ng/oocyte) reduced the current induced by adenosine to 27 ± 23% (mean ± S.D., 9 oocytes, 7 frogs, the range was 10–50%) of the control current. PKI-III (120 and 300 ng/oocyte) reduced the current induced by adenosine to 10 and 5% of control in the two cells tested. Since the inhibition induced by PKI injections was virtually irreversible under the conditions of the experiment (up to 3 hrs), two or three identical consecutive responses to adenosine were recorded before the injection of PKI to ensure the reproducibility of the response to adenosine in the cell chosen for injection. The quantity of injected PKI required for significant inhibition of the adenosine response varied considerably among the different oocytes. In the same oocyte, however, dose dependency could be clearly demonstrated (see Fig. 7). The inhibition produced by an injection of PKI developed fully during 20–60 min following the injection. Incubation of PKI-II with trypsin (2 mg/ml, 30–60 min at 30°) resulted in a reduction of the adenosine current only to 80 ± 16% of control (mean ± S.D., 7 oocytes, 6 frogs). The injection of an inert protein (bovine serum albumin, 450 and 600 ng/oocyte, 2 cells) had no effect on the adenosine response.

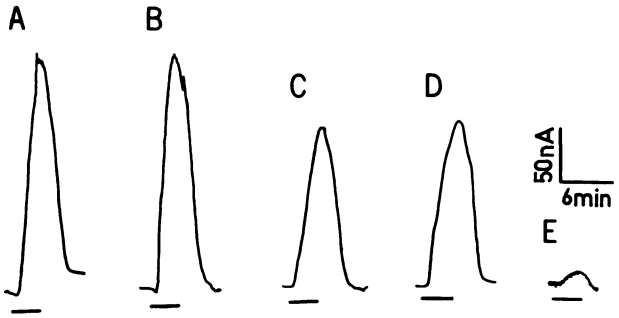

FIG. 7. *Effect of injected PKI-type III on the adenosine response in a single oocyte*

The membrane potential was clamped at −27 mV. A, B). Two consecutive control adenosine responses. C, D). Two consecutive adenosine responses following the injection of 30 ng of PKI (see "Experimental Procedures"). E). Adenosine response following the cumulative injection of 330 ng of PKI. The cumulative injection of intermediate PKI concentrations (90 and 150 ng) resulted in an intermediate inhibition (40 and 60%, respectively) of adenosine response (not shown). Adenosine (10^{-5} M, *bars* under each *trace*) was applied at 30-min intervals.

Interaction between the adenosine H and cAMP currents. When the adenosine H current was elicited on a background of a small cAMP-induced current (up to 50 nA), the two currents were additive (not shown). However, in the presence of a large cAMP-induced current, the H current was inhibited to 20–70% of control (10 oocytes, 9 frogs), depending on the cAMP "background"

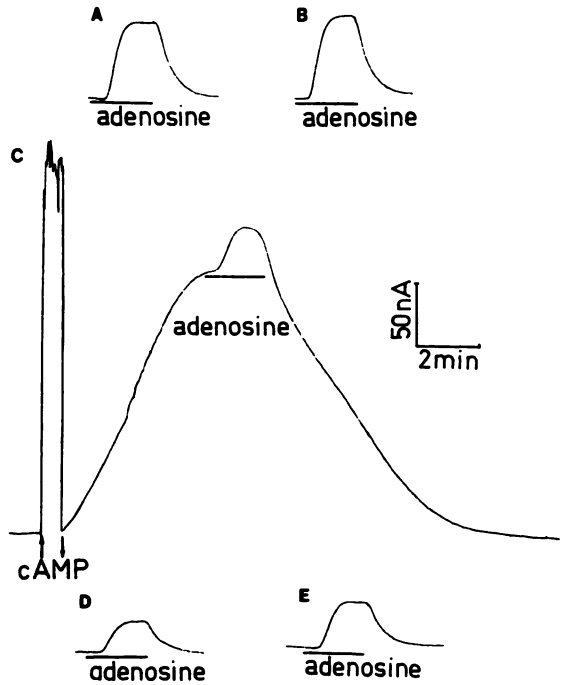

FIG. 8. *Large cAMP response induces cross-inhibition of H response elicited by adenosine*

The oocyte was clamped at −70 mV; the concentration of adenosine was 0.5 μM. A, B). Two consecutive control responses to adenosine. C). Iontophoretic injection of cAMP evoked a large response on top of which the H response elicited by adenosine was reduced. D, E). H responses elicited by adenosine 15 and 180 min after the peak of the cAMP response, respectively.

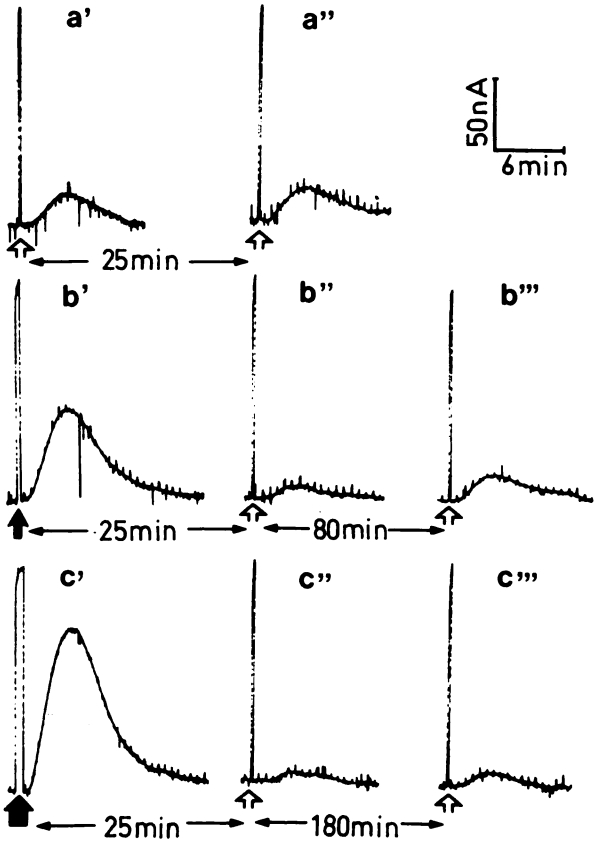

FIG. 9. *cAMP induces dose- and time-dependent inhibition of subsequent cAMP response in a single oocyte*

The oocyte was clamped at −70 mV. *Open arrows* stand for constant test iontophoretic pulses of cAMP. a', a''). A test response was reproducible 25 min following the preceding conditioning response. b', b'', b'''). A test response (b'') was reduced 25 min following the preceding larger conditioning cAMP response (b') but recovered after 80 min (b'''). c', c'', c'''). A test response (c'') was very much reduced 25 min following a preceding very large cAMP response (c') and only slightly recovered after 180 min (c''').

current amplitude, and recovered only partially (up to 3 hrs, see Fig. 8).

A conditioning cAMP-induced current (over 50 nA) caused a similar inhibition (in the range of 20–70%; 6 oocytes, 5 frogs) of the current induced by a subsequent cAMP application (test current). A representative experiment is shown in Fig. 9. Here the effect of a conditioning cAMP current on a small test cAMP current was studied. In Fig. 9 (a′, a′′), the conditioning and test currents were identical. When the conditioning cAMP current increased (Fig. 9, b′, c′) the test current was inhibited (Fig. 9, b′′, c′′). The extent of inhibition of the test current and the time course of its recovery depended on the amplitude of the conditioning current. When this current was sufficiently large (usually >100 nA at −50 mV, depending on the frog), complete recovery could not be achieved within 3 hrs following the conditioning cAMP current.

The converse effect of adenosine H current on the cAMP current was also studied. Relatively large adenosine-induced H currents (up to 300 nA) did not affect the subsequent cAMP currents (8 oocytes, 6 frogs). However, H currents induced by very large doses of adenosine (0.6 mM) could inhibit to some extent subsequent cAMP currents but only in some oocytes (4 out of 10 oocytes tested, 4 frogs).

DISCUSSION

In the present paper we demonstrate that the dominant adenosine response in *Xenopus* oocytes is a late slow hyperpolarization produced by an outward K^+ current (H current). Adenosine-evoked hyperpolarization has been shown to be associated with increased membrane conductance to K^+ ions in heart cells (10, 27) and in hippocampal neurons (11).

The H current is ascribed to the activation of Ra purine receptors (18), being dose dependent and saturable. The experimental data are consistent with a model in which the response is a linear function of $(\text{occupancy})^3$ (see "Results"). The phenomenological interpretation of this modification of positive cooperativity is not yet clear, but it implies that the H current can be generated only when three purine receptor sites are occupied concomitantly by an agonist. A similar model was proposed in order to account for the effect of calcium in transmitter release (28).

In this paper we also describe the *Xenopus* oocyte's membrane response to intracellular injection of cAMP. The cAMP response is a dose-dependent long-lasting outward current, carried mostly by K^+ ions. It is potentiated in the presence of extracellular phosphodiesterase inhibitors, further strengthening the role of cAMP in this process.

There is much similarity between the current evoked by injected cAMP and that evoked by extracellular application of adenosine; both currents arise from an increase in K^+ conductance and have a similar time course. This observation enhanced by the fact that the purine receptor involved is of the Ra type, known to activate adenylate cyclases in other biological systems (19), suggests that the purinergic H current in the oocyte may be mediated by an increase in the intracellular cAMP level. To support this conclusion, we have shown that the adenosine response satisfies the following additional requirements.

1. Early after the addition of adenosine and within the latency period of the electrophysiological response, direct measurements of intracellular cAMP concentrations reveal a moderate but consistent increase. On the average, the concentration of intracellular cAMP is increased by only 0.4 pmol/oocyte, but the injection of an even smaller quantity of cAMP (~0.15 pmol/oocyte) is sufficient to produce a measurable electrophysiological response (of several nA).

2. Although the adenosine-induced H current can be inhibited by *extracellular* theophylline due to a direct receptor blockade, it also can be potentiated by *intracellular* injection of theophylline, presumably due to a decreased rate of cAMP degradation.

3. Injection of PKI results in an attenuation of the adenosine current, implying that a cAMP-dependent protein kinase activation is involved. This result is similar to those observed with other cAMP-dependent membrane responses, such as the serotonin-induced increase in K^+ conductance in *Aplysia* neurons (29) and the Na^+ efflux in barnacle muscle fibers (30).

4. The cross-inhibition between the cAMP and H currents implies that the two systems converge upon one or more common mechanisms. This is the usual interpretation of the cross-inhibition observed between hormones (*e.g.* Refs. 31–33). In the present case, cross-inhibition can be ascribed to an increased level of intracellular cAMP, as a similar inhibition is induced between two cAMP currents. One possible mechanistic approach to explain cAMP-induced inhibition may be based on the demonstration (34) of a sustained stimulation of cAMP phosphodiesterase following a rise of intracellular cAMP levels.

Our results strongly indicate that, in *Xenopus* oocytes, cAMP serves as a second messenger in the chain of events that lead to the adenosine-induced opening of K^+ channels. This is the first demonstration of a purinergic electrophysiological response mediated by cAMP. Since the biological action of cAMP has been generally associated with the modulation of protein phosphorylations, it would be of interest to study such phosphorylations in the *Xenopus* oocyte system.

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Send reprint requests to: Dr. Yoram Lass, The Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv 69978, Israel.