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Hormone-regulated K^+ channels in follicle-enclosed oocytes are activated by vasorelaxing K^+ channel openers and blocked by antidiabetic sulfonylureas

(K^+ conductance/follicular cells/*Xenopus* oocyte/gonadotropin/cromakalim)

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ABSTRACT **Follicular oocytes from *Xenopus laevis* contain K^+ channels activated by members of the recently recognized class of vasorelaxants that include cromakalim and pinacidil and blocked by antidiabetic sulfonylureas, such as glibenclamide. These channels are situated on the adherent follicular cells and are not present in denuded oocytes. Cromakalim-activated K^+ channels are also activated by increases in intracellular cAMP, and cAMP-activated K^+ channels are blocked by glibenclamide. Although cromakalim and cAMP effects are synergistic, cromakalim activation of K^+ channels is drastically reduced or abolished by treatments that stimulate protein kinase C (e.g., muscarinic effectors, phorbol esters). Gonadotropins, known to play an essential role in ovarian physiology, also activate cromakalim and sulfonylurea-sensitive K^+ channels. Follicular oocytes constitute an excellent system for studying regulation of cromakalim-sensitive K^+ channels that are important in relation to a variety of disease processes, such as cardiovascular dysfunction and asthma, as well as brain function.**

Potassium channels are involved in a wide variety of biological functions (1). Therefore, it is not surprising that their pharmacology has attracted considerable interest in the past few years (2–4), which has led to the recent development of K^+ channel effectors of considerable importance for present and future therapeutic use. Two classes of such agents are antidiabetic sulfonylureas, which block ATP-sensitive K^+ (K_{ATP}) channels in beta pancreatic cells (3–6) and provoke insulin release (6, 7), and K^+ channel openers (KCOs), which hyperpolarize smooth muscle cells (3, 4, 8–12) and have important potential applications in a variety of diseases (3, 4, 8, 13), including hypertension and asthma. The relaxant effects of KCOs are antagonized by sulfonylureas (3, 4, 8, 12), and both families of drugs have the same target channel (3, 4, 8, 12, 14).

Potassium channels sensitive to both KCOs and to sulfonylureas are also present in the brain (15, 16), where they play diverse important functions (16), including key roles in glucose-regulated neurosecretion and in disorders associated with anoxia and ischemia (16, 17). KCOs have been found to have antiepileptic effects (18, 19) as well.

In follicle-enclosed oocytes from *Xenopus laevis*, endogeneous K^+ channels have been demonstrated (20). This system provides a valuable model for investigating K^+ channel regulation because K^+ channel activity in follicular oocytes is modulated by cAMP, by protein kinase C-dependent processes, and by a large variety of hormones and transmitters, such as catecholamines, adenosine, gonadotropins, vasoactive intestinal peptide, the E series of prostaglandins, atrial natriuretic factor, and muscarinic agonists (20–24).

This paper shows that follicular oocytes from *X. laevis* have a K^+ channel that is activated by smooth muscle cell KCOs and blocked by antidiabetic sulfonylureas. Moreover, this channel is modulated both by protein kinase C and by variations in intracellular cAMP.

MATERIALS AND METHODS

Clusters of oocytes were removed surgically from adult *X. laevis* females under tricaine anesthesia and were stored in Barth saline solution [88 mM NaCl/1 mM KCl/0.82 mM $MgSO_4$/0.33 mM $Ca(NO_3)_2$/0.41 mM $CaCl_2$/2.4 mM $NaHCO_3$/10 mM Hepes, pH 7.4 with NaOH] supplemented with penicillin at 100 international units per ml and streptomycin at 100 μg/ml. Oocytes were defolliculated manually in a glass Petri dish in Barth medium (24). Oocyte diameters were measured in at least two directions (at 90°) in case they were not strictly spherical. Oocytes were impaled with two glass microelectrodes filled with 3 M KCl (0.5- to 2.0-MΩ resistance) and were voltage-clamped using the Dagan 8500 voltage clamp amplifier. Voltage commands were generated using PCLAMP (Axon Instruments, Burlingame, CA) running on an IBM PC/AT computer.

The oocytes were routinely voltage-clamped at −20 mV, which is the predicted equilibrium potential for chloride ions. This protocol tended to minimize any eventual influence of chloride in the ionic currents recorded. Drugs were applied externally by addition to the perfusate by means of a peristaltic pump at a flow rate of 3 ml/min. Saline solution (ND 96) of the following composition was used in all procedures unless otherwise stated: 96 mM NaCl/2 mM KCl/1.8 mM $CaCl_2$/2 mM $MgCl_2$/5 mM Hepes, pH 7.4 with NaOH. All chemicals were obtained from Sigma, unless otherwise stated. The variability of the results was expressed as the SE of the mean; *n* indicates the number of cells contributing to the mean, and *N* indicates the number of *Xenopus*.

RESULTS AND DISCUSSION

Fig. 1*A* shows that the KCO cromakalim (Ck), also called BRL34915, induces a large hyperpolarization of the membrane potential of follicular oocytes. Control oocytes have a mean resting membrane potential of −57 ± 6 mV (n = 8, N = 3), whereas Ck (100 μM)-treated oocytes have a membrane potential of −88 ± 8 mV (n = 7, N = 3). The Ck-induced

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Abbreviations: K_{ATP}, ATP-sensitive K^+ channels; KCO, K^+ channel opener; Ck, cromakalim; hCG, human chorionic gonadotropin; 8-Br-cAMP, 8-bromoadenosine 3′,5′-cyclic monophosphate; I_{cAMP}, cAMP-activated K^+ conductance.

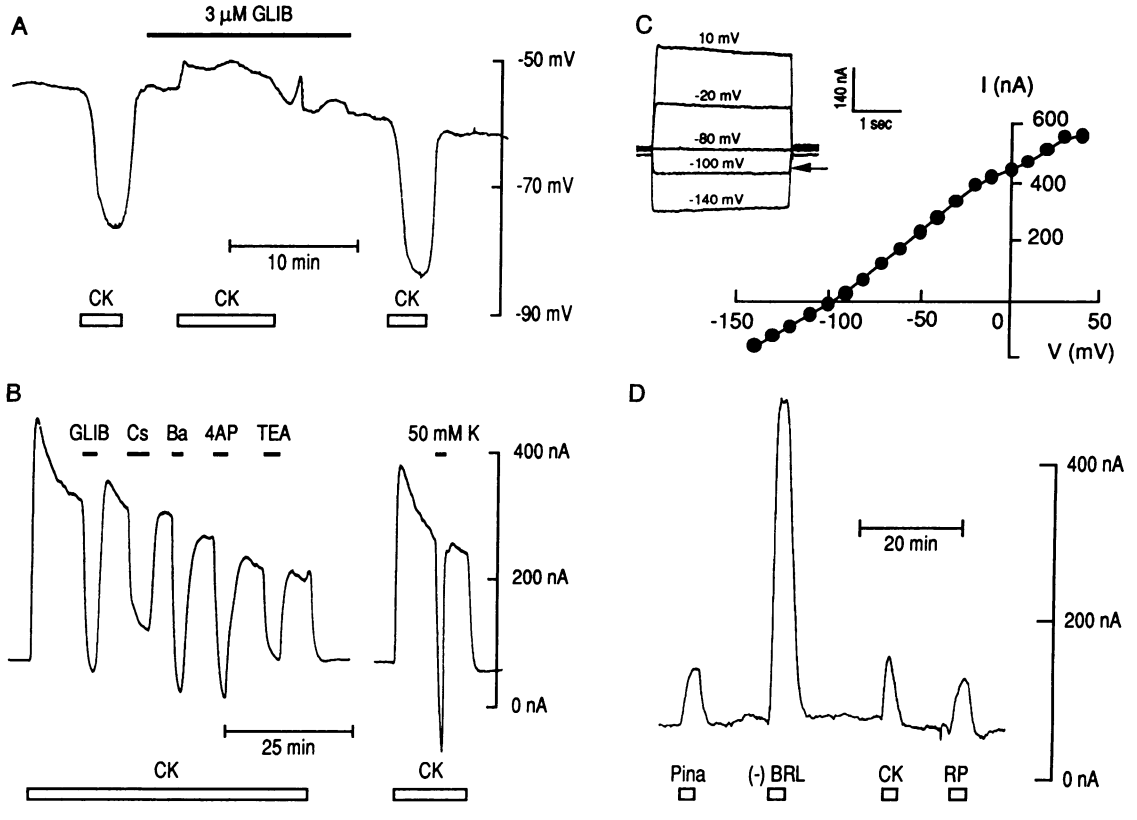

FIG. 1. Electrogenic effects of Ck (CK) in follicle-enclosed oocytes from *X. laevis*. (*A*) Ck-induced hyperpolarization is blocked by glibenclamide (GLIB) (Ck, 100 μM; glibenclamide, 3 μm). The glibenclamide block is reversible upon washing. (*B*) Ck (100 μM) elicits an outward current that is blocked by glibenclamide (2 μM), Cs^+ (20 mM), Ba^{2+} (2 mM), 4-aminopyridine (4AP; 2 mM), and tetraethylammonium (TEA; 30 mM). Outward current was reversed at 50 mM external K^+. (*C*) Voltage dependence of Ck (100 μM)-induced currents. Control traces were digitally subtracted. Voltage steps of 4 s in duration were elicited every 20 s from −80 mV. (*D*) Comparative outward currents induced by 100 μM pinacidil (Pina), (−)BRL38227, (+)BRL34915 (CK), and RP61419 (RP).

hyperpolarization is reversibly abolished by the potent antidiabetic sulfonylurea glibenclamide (5).

The Ck-induced hyperpolarization is associated with the generation of a slow outward current (Fig. 1*B*). This current is reversibly blocked by glibenclamide, and it is also blocked by Cs^{2+}, Ba^{2+}, tetraethylammonium, and 4-aminopyridine, which are also known to inhibit glibenclamide-sensitive K^+ channels (4, 12). It is unaffected by toxins such as apamin (1 μM), dendrotoxin I (1 μM), or charybdotoxin (1 μM), which are blockers of other types of Ca^{2+}-dependent and voltage-dependent K^+ channels (2) but are without effect on glibenclamide-sensitive K^+ channels (4, 12). The outward current elicited by Ck was reversed (Fig. 1*B*) when the external K^+ concentration was raised to 50 mM, also suggesting that the Ck-induced current is a K^+ current.

The current–voltage relationship of the Ck-induced current is presented in Fig. 1*C*. Current reversal is observed at −92 ± 3 mV (n = 7, N = 2), which is the expected reversal potential for a K^+ current, thus confirming that the Ck-induced current is carried by K^+ ions.

Besides Ck, there now exists a whole variety of other interesting vascular KCOs (3, 4, 8, 25), including pinacidil, nicorandil, RP61419, and minoxidil sulfate. The comparative activity of some of these different types of compounds on K^+ channels in follicle-enclosed oocytes is presented in Fig. 1*D*. Pinacidil and RP61419 are as effective as Ck. The Ck (3*S*,4*R*) enantiomer (−)BRL38227 is about five times more potent than Ck itself. (+)BRL38226 has no K^+ channel-opening activity. This stereoselectivity is the same as that observed in blood pressure lowering (4). Minoxidil sulfate and nicorandil are inactive, unlike in vascular tissue.

The Ck response varies with the follicular stage of development. Small responses are first detected in late-stage IV follicles, and the response then increases considerably in stages V and VI (Fig. 2*A*). Developmental properties of the Ck-induced response are paralleled by developmental changes in the K^+ response induced in follicular oocytes by cAMP injection or by gonadotropins (20, 21, 24). The K^+ response produced by intraoocyte cAMP injection or by the various hormones that increase cAMP is known to be abolished in defolliculated oocytes (24), indicating that cAMP-activated K^+ currents arise in follicular cells and can be monitored within the oocyte through electrical coupling by gap junctions (20, 24). A comparison of outward K^+ currents elicited by cAMP, human chorionic gonadotropin (hCG), and Ck in the same follicle-enclosed oocyte at stage V is presented in Fig. 2*B*. Both the Ck activation and K^+ channel responses produced by cAMP and hCG are abolished in defolliculated oocytes (n = 9, N = 2) (Fig. 2*C*). In denuded oocytes, observed inhibitory effect of Ck is due to an inhibition of a K^+ current in the oocyte itself by the (+)enantiomer of Ck (unpublished work). These results indicate that, similarly to the cAMP-induced K^+ current, the Ck-induced K^+ current arises in follicular cells and not in the oocyte itself.

Application of progesterone to fully grown unovulated oocytes is known to induce ovulation *in vitro* (26). Treatment of unovulated oocytes (stage V) with progesterone (1 μM for 12 hr) leads to maturation. Mature follicular oocytes have a resting membrane potential of −23 ± 6 mV (n = 7, N = 2) and do not respond to either cAMP, hCG, or Ck (Fig. 2*D*).

The concentration-dependence of the inhibition of the Ck-induced K^+ current by glibenclamide is presented in Fig.

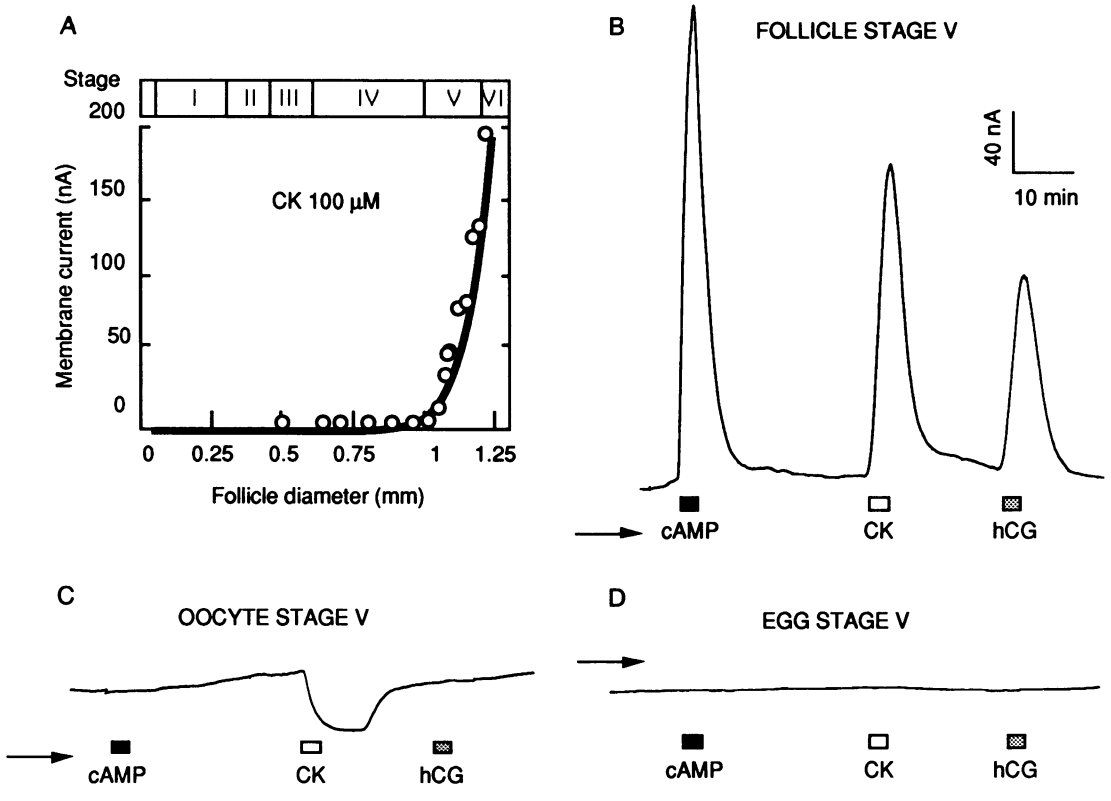

FIG. 2. (*A*) Relationship between follicle diameter or stage of development and the peak amplitude of the response to Ck (100 μM; CK). All follicles were isolated from the same frog and were analyzed for the Ck effect on the same day at a holding potential of −20 mV. (*B*) Comparative responses obtained with 8-bromoadenosine 3′,5′-cyclic monophosphate (8-Br-cAMP; 300 μM), Ck (100 μM), and hCG (5 international units/ml) in a single follicle-enclosed oocyte at stage V. The oocyte was clamped at −20 mV. (*C*) Same protocol on a defolliculated stage V oocyte. (*D*) Same protocol on a follicle-enclosed oocyte treated for 12 hr with 1 μM progesterone.

3*B*. Half-maximal inhibition is observed at 200 nM glibenclamide. Other antidiabetic sulfonylureas also depress the Ck response. Glipizide is as potent as glibenclamide. However, tolbutamide, which must be used in higher therapeutic concentrations and is known to be much less active on K_{ATP} channels in beta-pancreatic cells (5), does not inhibit Ck effects at 10 μM and is only a partial inhibitor (≈40%) at concentrations as high as 100 μM (data not shown).

Fig. 3 *A* and *B* shows that the cAMP-activated K^+ conductance (I_{cAMP}) of follicular cells is inhibited (≈60%) by glibenclamide. The half-maximal inhibition of I_{cAMP} produced by glibenclamide was observed at ≈ 100 nM (Fig. 3*B*). Consequently, dose–response curves for glibenclamide inhibition are very similar for Ck-activated and cAMP-activated K^+ channels. Glibenclamide also potently inhibited the K^+ current induced by forskolin (which directly activates adenyl cyclase), or by hCG (Fig. 3*C*).

These observations suggest that there are at least two classes of K^+ currents activated by cAMP in follicular oocytes and that one of them, which constitutes most of the cAMP-dependent response, corresponds to glibenclamide-sensitive K^+ channels.

At this stage it seemed important to analyze the sensitivity of the Ck-sensitive K^+ channel to cAMP. Fig. 4 shows that the Ck response is greatly potentiated when oocytes have been submitted to treatments that increase their intracellular cAMP concentration. For example, 3-isobutyl-1-methylxanthine, a phosphodiesterase inhibitor that inhibits cAMP degradation (Fig. 4*B*), and low concentrations of forskolin, which activates adenyl cyclase (Fig. 4*D*), increase intracellular cAMP but not to a point at which one can detect significant I_{cAMP} values. However, this cAMP increase is high enough to provoke an important potentiation of the Ck effect. The potentiation is even higher when the intracellular cAMP concentration of follicular oocytes is increased by treatment with 8-Br-cAMP) (Fig. 4*A*) or hCG (Fig. 4*C*).

This series of experiments suggests that low doses of cAMP "prime" the K^+ channels in the membrane, so they can be opened more easily by Ck.

Acetylcholine acting upon muscarinic receptors attenuates the I_{cAMP} elicited by adrenaline, dopamine, adenosine, gonadotropins, vasoactive intestinal peptide, forskolin, prostaglandins, and intracellular injections of cAMP (20, 22, 27). Muscarinic attenuation of K^+ currents is mimicked by phorbol esters (27), and both effects are mediated through activation of protein kinase C (27). Fig. 4 *E* and *F* shows that the Ck response is also inhibited by both acetylcholine [which activates by itself a different K^+ current (28)] and phorbol 12-myristate-13-acetate.

All these results taken together indicate that the class of K^+ channels activated by compounds such as Ck, pinacidil, or RP61419 and blocked by sulfonylureas is the same as that which (*i*) is activated by hormones and transmitters that increase cAMP concentrations and (*ii*) is negatively modulated by factors that increase intracellular diacylglycerol levels and consequently stimulate protein kinase C activity.

Hormones and neurotransmitters that modulate K^+ channel activity in follicle-enclosed oocytes are also physiologically important in the regulation of smooth muscle contraction and for brain function. For example, follicular cells, similarly to vascular cells, contain receptors for potent vasoconstrictors, such as angiotensin II, which are coupled to phosphoinositol hydrolysis and Ca^{2+} mobilization (29), and for vasodilators, such as vasoactive intestinal peptide, which increase cAMP (23). It would then be expected that at least some of the conclusions of this paper may also hold for Ck-activated K^+ channels in both smooth muscle cells and neurons.

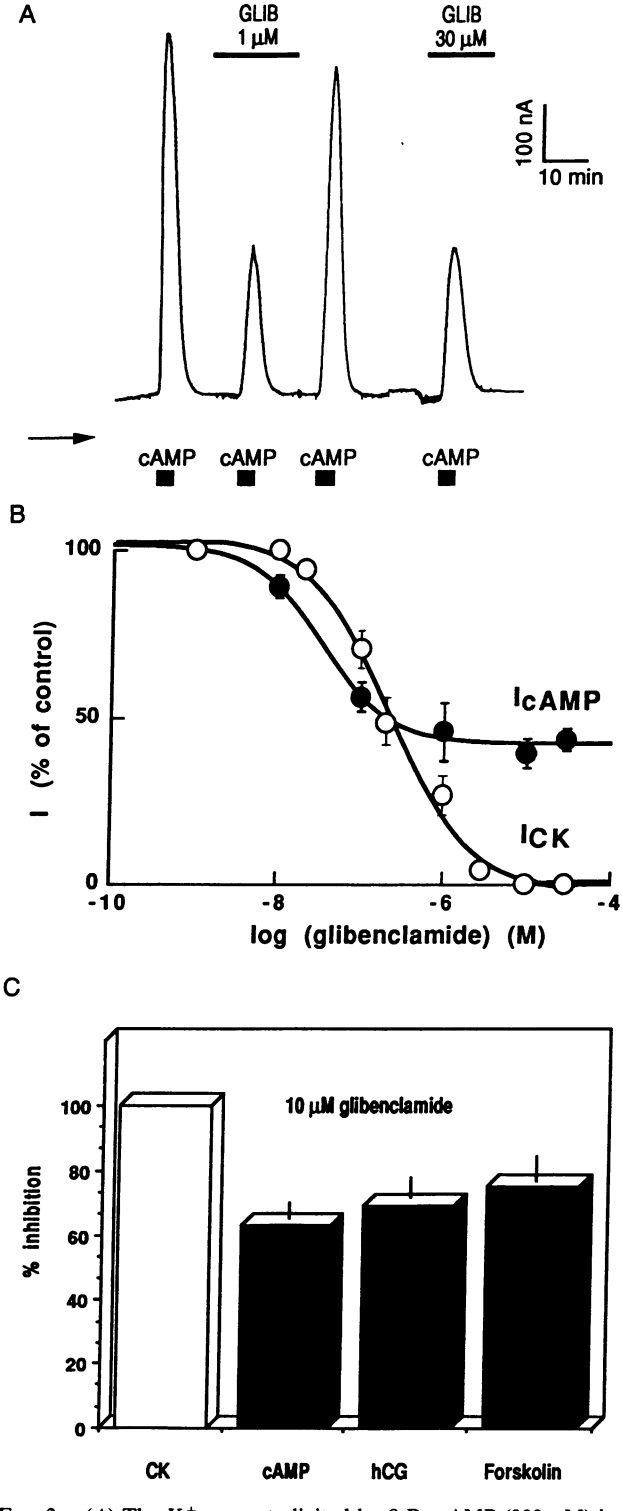

FIG. 3. (*A*) The K^+ current elicited by 8-Br-cAMP (300 μM) is inhibited by glibenclamide (GLIB). (*B*) Comparative dose–response curves for inhibition by glibenclamide of K^+ currents induced by 8-Br-cAMP (300 μM; I_{cAMP}) and by Ck (100 μM; I_{CK}). All follicles were isolated from the same frog and assayed within 2 days after isolation. (*C*) Comparative blockade (in % inhibition) by glibenclamide of K^+ currents elicited by treatment of follicular oocytes with Ck (100 μM; CK), 8-Br-cAMP (300 μM/cAMP), with hCG (5 international units/ml) and forskolin (2 μM). In all experiments the membrane potential was held at −20 mV. Seven oocytes were used for the determination of each mean data point.

Gonadotropins are important hormones involved in the regulation of various aspects of ovarian physiology (30). Because their electrophysiological effects are largely mimicked by KCO and blocked by sulfonylureas, these two categories of drugs might be expected to modulate growth and maturation and enlargement of the follicle as well as ovulation and meiotic division of the cell.

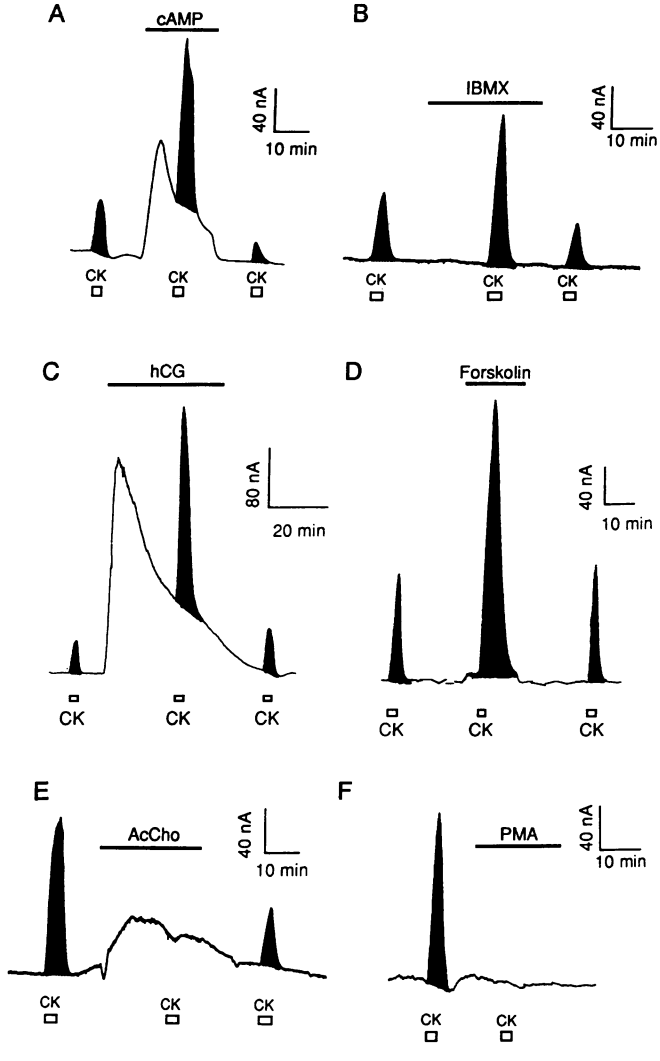

FIG. 4. (*A–D*) Treatments that increase intracellular cAMP concentrations potentiate Ck induction of an endogenous K^+ current. (*A*) 300 μM 8-Br-cAMP. (*B*) 500 μM 3-isobutyl-1-methylxanthine (IBMX). (*C*) hCG at 5 international units/ml. (*D*) 0.1 μM forskolin. Acetylcholine (AcCho; 10 μM) (*E*) or phorbol ester (PMA; 0.1 μM) (*F*) drastically reduce the Ck (100 μM) response. All experiments were done on a follicular oocyte clamped at −20 mV.

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