through *RAS* would be effected by a factor responsive to nutritional signals, which could facilitate exchange of guanine nucleotides on *RAS*. Although there is no evidence that such a factor acts on *RAS*, proteins providing guanine nucleotide exchange activity are known for hormone-responsive G proteins (*2*) and for translation factors EF-Tu and elF-2 (*21*). Like these proteins, *RAS* binds GDP and GTP with high affinity (*1*).

The only apparent biochemical difference between *RAS2* and *RAS2*^{val19} proteins is the reduction of GTPase activity of the latter. The active form of *RAS2*^{val19} would therefore have an extended half-life and could require less exchange factor activity to remain stimulatory. Alternatively, published data suggests that the GDP-bound form of *RAS2*^{val19} may be as active as the GTP adduct of *RAS2* (*10*), which would also reduce the need for GTP-GDP exchange. The ability of the activated form of *RAS2* to suppress the lethality of the *cdc25* disruption allele therefore raises the possibility that *CDC25* could act as a guanine nucleotide exchange factor for *RAS*. However, the sequence of *CDC25* has been determined, and a comparison of the predicted amino acid sequence with known proteins and translated open reading frames was reported to give no significant homologies (*15*). Direct evidence that the *CDC25* gene product functions as a modulator of *RAS* activity, whether as a GTP exchange factor or through an as yet unknown activity, awaits genetic and biochemical analysis of *CDC25* mutations.

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Xenopus Oocytes Injected with Rat Uterine RNA Express Very Slowly Activating Potassium Currents

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Under the influence of estrogen, uterine smooth muscle becomes highly excitable, generating spontaneous and prolonged bursts of action potentials. In a study of the mechanisms by which this transition in excitability occurs, polyadenylated RNA from the uteri of estrogen-treated rats was injected into *Xenopus* oocytes. The injected oocytes expressed a novel voltage-dependent potassium current. This current was not observed in oocytes injected with RNA from several other excitable tissues, including rat brain and uterine smooth muscle from ovariectomized rats not treated with estrogen. The activation of this current on depolarization was exceptionally slow, particularly for depolarizations from relatively negative membrane potentials. Such a slowly activating channel may play an important role in the slow, repetitive bursts of action potentials in the myometrium.

THE SMOOTH MUSCLE OF THE UTERUS, the myometrium, in common with many neuronal systems (*1*), undergoes long-lasting changes in electrical excitability. Estrogen treatment of the adult uterus causes growth of the myometrium and the appearance of spontaneous electrical activity consisting of prolonged bursts of action potential firing lasting many seconds (*2*). Little is known about the ionic mechanisms that underlie such slow electrical events, in part because of the technical difficulties associated with studying smooth muscle cells. We have used the *Xenopus* oocyte translation system (*3–6*) to detect mRNA (messenger RNA) coding for ion channels in uterine smooth muscle. Previous work by Dahl and co-workers (*6*) has shown that RNA from uterus can be used to express gap-junction proteins. We here describe a novel voltage-dependent potassium current observed in frog oocytes injected with polyadenylated [poly(A)$^+$] RNA from the uterine horns of rats treated with estrogen.

Actively cycling, adult female Sprague-Dawley rats (Charles River) were ovariectomized and implanted with Silastic capsules containing 100% β-estradiol (*7*). After 3 days, the animals were killed, and RNA was prepared from the uterine horns (*8*). *Xenopus* oocytes were injected with 200 ng of poly(A)$^+$ RNA in 50 nl of sterile water. Control oocytes were injected with 50 nl of sterile water. After incubation for 3 days the oocytes were voltage-clamped at 20° to 23°C by the use of conventional two-microelectrode techniques (*9*).

Figure 1 shows voltage-clamp records obtained from an oocyte 3 days after injection of 200 ng of uterine poly(A)$^+$ RNA. Depolarizations from a holding potential of −40 mV elicited a voltage- and time-dependent outward current, first evident at −30 mV. On repolarization to −40 mV, large, slowly decaying outward tail currents were observed. Similar outward currents were re-

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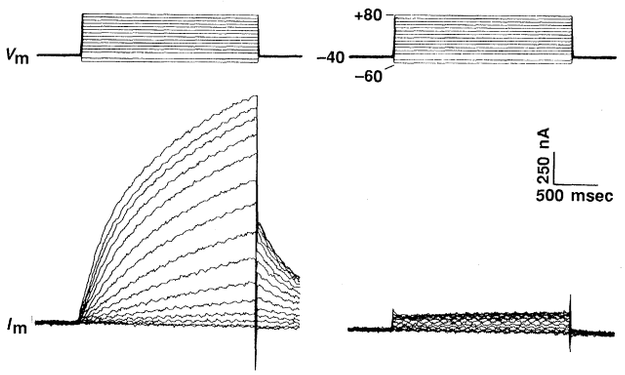

Fig. 1. Voltage-dependent current (I_m) in oocytes injected with uterine RNA (left) or with water (right). The oocytes were held at −40 mV and depolarized or hyperpolarized stepwise to test potentials between −60 and +80 mV for 2 seconds. Leakage currents were subtracted from the records by appropriate multiples of the current elicited by a hyperpolarizing pulse to −50 mV. The low-chloride bathing medium consisted of 96 m*M* sodium aspartate, 2 m*M* potassium aspartate, 1.8 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, and 5 m*M* Hepes [4-(2-hydroxyethyl)-1-pipe-razineethanesulfonic acid], *p*H 7.6. The resting potential of the RNA-injected oocyte was −46 mV, and its input resistance was 0.2 megohm. The resting potential of the water-injected control was −42 mV, and its input resistance was 0.2 megohm. The mean resting potentials (−45 ± 2 mV) and input resistances (0.5 ± 0.1 megohm) of the RNA-injected oocytes expressing this current did not differ significantly from the control values of −42 ± 1 mV and 0.3 ± 0.1 megohm (water-injected oocytes, n = 4, from the same frogs). V_m, membrane potential.

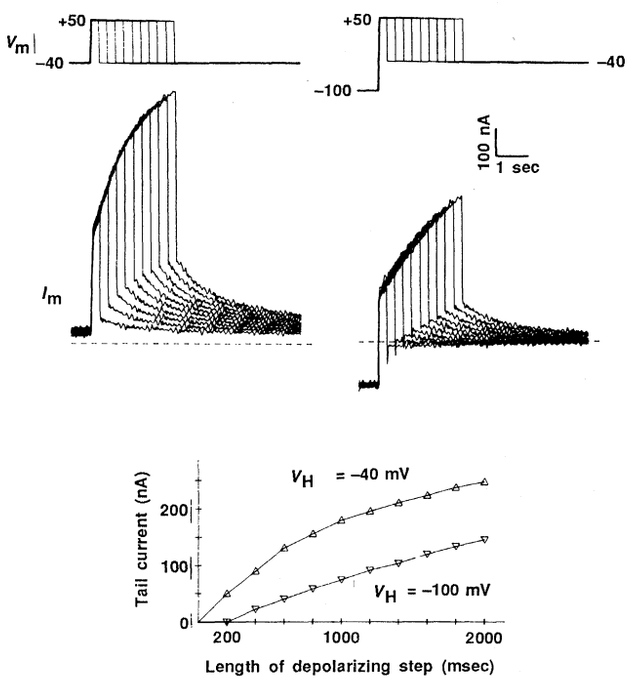

Fig. 2. Effect of holding potential (V_H) on activation of the outward current. The cell was held at −40 mV (left) or −100 mV (right) and stepped to +50 mV for durations in a sequence between 200 msec and 2 seconds to produce an envelope of tail currents. The current records in this figure and Figs. 3 and 4 have not been corrected for leakage; the zero current level is indicated by a dashed line in each figure. The bottom graph shows tail current amplitudes plotted as a function of the duration of the depolarizing step from a holding potential of −40 (△) or −100 mV (▽). The bath solution contained 96 m*M* NaCl, 2 m*M* KCl, 1.8 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, and 5 m*M* Hepes, *p*H 7.6. Figures 2, 3, and 4 show data from the same cell, which had a resting potential of −50 mV and an input resistance of 0.5 megohm.

corded in 9 out of 12 oocytes injected with uterine RNA. (The oocytes were from five different frogs.) The mean outward tail current measured at −40 mV after a 2-second pulse to +50 mV was 227 ± 66 nA (SEM).

Figure 1 also shows records from a water-injected control oocyte (from the same frog as the RNA-injected cell). Currents similar to the slow outward current observed in uterine RNA–injected oocytes were never seen in other oocytes (>20 frogs), including noninjected oocytes (n = 124), water-injected oocytes (n = 21), and oocytes injected with RNA from the central nervous systems of male rats (n = 4), male or female C57Bl/6 mice (n = 6), *Aplysia californica* (n = 113), and *Helix aspersa* (n = 11). In particular, the current described here differs from voltage-dependent K^+ currents expressed in oocytes injected with poly(A)$^+$ RNA from rat brain, which activate at a rate about two orders of magnitude faster than those in oocytes injected with rat uterine RNA and which undergo inactivation (*5*). We were also unable to detect the slowly activating current in oocytes injected with RNA from the relatively unexcitable uterine smooth muscle of ovariectomized animals not given estrogen. Although this result suggests that the RNA species that determines the expression of this current may be regulated by estrogen, we cannot exclude the possibility that the tissue that was not treated with estrogen contained other factors that interfered with the expression of this current in the oocytes.

The time course of expression of the new current after RNA injection was examined in one experiment with four cells. In two cells the current could be detected after 2 days of incubation, and had increased an additional 30% by day 3. In the other two oocytes the slow current was detected only after 3 days of incubation. This time course is comparable to that described for the translation of other ion channel proteins in oocytes (*4*, *5*).

The RNA-induced current activates unusually slowly. The amplitudes of the current evoked by a depolarizing step and the accompanying tail currents increased with the duration of the test step (Fig. 2). Activation of the underlying conductance did not appear to be complete after 2 seconds at +50 mV. The effects of longer depolarizations proved difficult to study because very large (endogenous) time-dependent inward currents are elicited by more prolonged depolarizations of the oocyte (*10*). The slow outward current could be reduced by holding the membrane potential at a more negative value (Fig. 2, right). The current activated more slowly from a holding potential of −100 mV than from −40 mV. The effect was reversed by returning to the more positive holding potential of −40 mV. Intermediate effects were seen with holding potentials of −60 and −80 mV.

The slowly activating current was carried mainly by potassium ions (Fig. 3). In external medium containing 2 m*M* K^+, the reversal of the tail currents occurred at −93 ± 4 mV (n = 4), close to the estimated reversal potential of −102 mV for K^+ in *Xenopus* oocytes (*11*). When K^+ concentration was increased to 22 m*M*, the tail currents reversed at about −35 mV, in reasonable agreement with the shift of 60 mV predicted by the Nernst equation for K^+. In addition, the current could be reduced by the K^+ channel blockers tetraethylammonium (10 to 20 m*M*, two cells) and Cs^+ (3 m*M*, one cell). It is unlikely that chloride ions contribute significantly to the slowly activating current, since the Cl^- equilibrium potential is about −24 mV for *Xenopus* oocytes in normal bathing solution (*11*); moreover, the characteristics of this current were similar in media deficient in Cl^- (Fig. 1) and normal media containing Cl^- (Figs. 2, 3, and 4). The reversal potential for the current was insensitive to changes in Cl^- concentration.

This current differs from many slowly activating K^+ currents that require previous

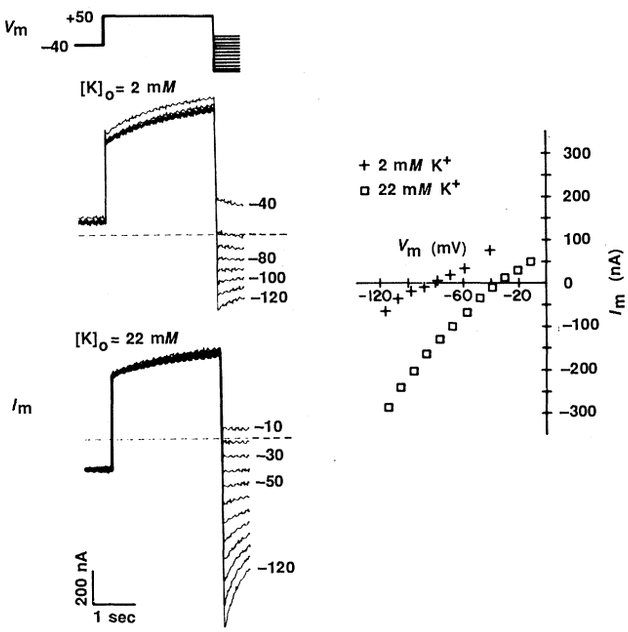

Fig. 3. Dependence of the reversal potential for slow outward tail currents on the external potassium concentration. The cell was held at −40 mV (left, upper traces), depolarized to +50 mV for 2 seconds, and then repolarized to various potentials. The currents were recorded in either 2 m*M* $[K]_o$ (middle traces, same bath solution as Fig. 2) or 22 m*M* $[K]_o$ (lower traces, 20 m*M* KCl added to the bath). (Right) The amplitudes of the tail currents are plotted as a function of voltage in 2 m*M* K^+ (+) or 22 m*M* K^+ (□). The amplitudes were measured by subtracting the leakage current from the initial tail current.

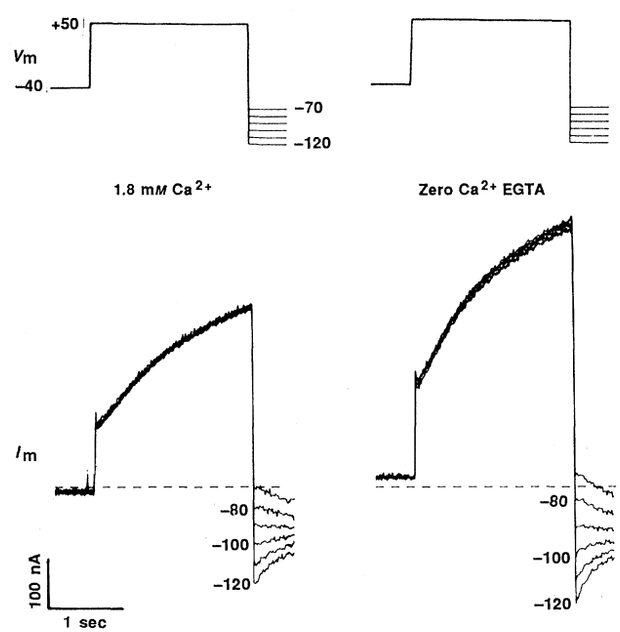

Fig. 4. Effect of reduced external calcium concentration on the slow outward current. The sequence of voltage-clamp commands was as for Fig. 3. The traces show currents in normal calcium-containing medium (same as in Fig. 2) and in a modified solution containing no added calcium, 2.8 m*M* $MgCl_2$, and 0.1 m*M* EGTA.

entry and accumulation of free calcium ions (*12*). The tail currents (Fig. 4) were not blocked in a Ca^{2+}-deficient, EGTA-containing medium (n = 3), even after 15 to 20 minutes. In two of the cells, the amplitudes of the current during the depolarizing pulse and of the tail currents showed a modest increase during exposure to the Ca^{2+}-deficient medium and then returned to control levels in medium containing 1.8 m*M* Ca^{2+}. In the Ca^{2+}-deficient medium the endogenous calcium-activated chloride current (*13*) was, by contrast, blocked, which indicated that calcium entry had been suppressed.

The reduced activation of the current from relatively negative holding potentials (Fig. 2) also persisted in the Ca^{2+}-deficient, EGTA-containing medium and was therefore not triggered by calcium entry. It seems plausible that the inhibition is an effect of membrane potential per se, reminiscent of, although apparently much larger than, effects of prehyperpolarization described for the delayed rectifier K^+ current in nerve (*14*). The reduced activation of the current may reflect voltage-induced shifts in the occupancy of various closed states of the underlying ion channel.

The simplest explanation for the appearance of this K^+ current in oocytes injected with RNA from the uterus is that uterine RNA, in contrast to other excitable tissues, contains significant amounts of an mRNA species that codes for this novel K^+ channel. Because myometrial smooth muscle is the major tissue component of the uterus, it seems most likely that the active RNA species originates in this smooth muscle. Electrophysiological studies of uterine smooth muscle preparations have, thus far, only been able to characterize the rapidly activating K^+ conductances (*15*) similar to those present in other excitable tissues. Single-channel recording with the use of cell-free patches from dissociated longitudinal smooth muscle of the rabbit jejunum has, however, provided evidence for a class of very slowly activating K^+ channels in smooth muscle cells (*16*). The voltage- and time-dependent properties of the slowly activating conductance we have described here may play an important role in shaping the slow electrical bursting of the myometrium. For example, the slow activation of this current during the maintained depolarization that accompanies a burst of action potentials could terminate the burst after many seconds.

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9. Before being injected, the oocytes were treated with collagenase (Boehringer Mannheim) at 2 mg/ml in a

nominally Ca^{2+}-free solution for 45 to 60 minutes to remove surrounding cells. After injection of RNA or water the oocytes were incubated in 70% L-15 medium (Gibco) containing penicillin (100 μg/ml) and streptomycin (100 U/ml) at room temperature (20° to 25°C). The oocytes were voltage-clamped at 20° to 23°C by the use of conventional two-microelectrode techniques.
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Coexistence of Guanylate Cyclase and Atrial Natriuretic Factor Receptor in a 180-kD Protein

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Atrial natriuretic factor (ANF) is a peptide hormone that is released from atria and regulates a number of physiological processes, including steroidogenesis in adrenal cortex and testes. The parallel stimulation of membrane guanylate cyclase and corticosterone production in isolated fasciculata cells of rat adrenal cortex has supported the hypothesis of a mediatory role for cyclic guanosine monophosphate (cyclic GMP) in signal transduction. A novel particulate guanylate cyclase tightly coupled with ANF receptor was purified approximately 273,000-fold by two-step affinity chromatography. The enzyme had a molecular size of 180 kilodaltons and was acidic in nature with a *p*I of 4.7. Its specific activity was 1800 nanomoles of cyclic GMP formed per minute per milligram of protein. The purified enzyme bound ANF with a specific binding activity of 4.01 nanomoles per milligram of protein, a value that is close to the theoretical binding activity of 5.55 nanomoles per milligram of protein for 1 mole of the ligand binding 1 mole of the receptor protein. These results indicate that the guanylate cyclase–coupled ANF receptor exists in a 180-kilodalton protein of rat adrenocortical carcinoma and represent a step toward the elucidation of the basic mechanism of cyclic GMP–mediated transmembrane signal transduction in response to a hormone.

STUDIES WITH ISOLATED FASCICULata cells of rat adrenal cortex and rat adrenocortical carcinoma indicated a physiological mediatory role for cyclic guanosine monophosphate (cyclic GMP) in steroidogenic signal transduction and led to the proposal of a hypothetical working model in which membrane guanylate cyclase was the key enzyme in receptor-mediated cyclic GMP signal pathway [reviewed in (*1*)]. Until recently a strong bias existed against the presence of a hormone-dependent membrane guanylate cyclase in any endocrine or nonendocrine tissue. The belief was that there was only one guanylate cyclase, a soluble enzyme, which was documented to be hormone-independent and nonspecifically activated by a variety of nitrite-generating compounds and agents that affect the oxidation-reduction potential of biological reactions (*2, 3*). These reservations were overcome by the demonstration of two distinct types of guanylate cyclase—membrane and soluble—in rat adrenal and rat adrenocortical carcinoma cells; only the membrane enzyme is hormone-specific (*4–6*).

More recently, the above results were corroborated in various rat tissues by demonstrating that atrial natriuretic factor (ANF) selectively stimulates particulate guanylate cyclase (*7*); these tissues included the rat adrenal gland (*8*). In vivo infusion studies with rat adrenal venous blood (*9*) and in situ studies with isolated fasciculata cells of rat adrenal cortex showed that ANF stimulates the production of corticosteroids (*10*). Similarly, in mouse interstitial (*11*) and Leydig cells (*12, 13*), testosterone production is increased by ANF. The mechanism of the ANF-dependent stimulation of steroidogenesis is not known, but the stimulation of membrane guanylate cyclase in parallel with the generation of an ANF-dependent steroidogenic signal suggested that this enzyme may have a role in mediating signal transduction (*10*).

Elucidating the biochemical mechanism of the mediatory role of cyclic GMP in receptor-mediated transmembrane signal transduction requires purification of the membrane guanylate cyclase. Only partial purification of any mammalian particulate guanylate cyclase has been achieved to date (*14*). We now describe purification of the membrane guanylate cylase and demonstrate that this enzyme is tightly coupled with the ANF receptor.

Membranes isolated from rat adrenocortical carcinoma cells were solubilized as in (*15*), adjusted to a final concentration of 5 m*M* $MnCl_2$, and adsorbed onto a guanosine triphosphate (GTP)–agarose affinity resin, which was suspended in and extensively washed with buffer A [25 m*M* triethanolamine hydrochloride (*p*H 7.6), 5 m*M* $MnCl_2$, and 1 m*M* 3-[(3-cholamidopropyl) - dimethylammonio] - 1 - propanesulfonate (CHAPS)] until there was no detectable protein (absorbance at 280 nm). The guanylate cyclase was eluted at room temperature with 25 m*M* triethanolamine (*p*H 7.6) 1 m*M* CHAPS, and 2 m*M* EDTA. The pooled enzymic fractions were adjusted to 5 m*M* Mn^{2+} and adsorbed to the cyclic GMP–Sepharose, which had been equilibrated with buffer A. The resin was loaded onto a small column (1.6 by 8 cm); flow-through was cycled back on the column once; and the column was washed extensively with buffer A. The enzyme was eluted with buffer A containing 2 m*M* EDTA (crossed affinity purification step) (lane 5 in Fig. 1A). The enzyme was thus purified approximately 273,000-fold.

The homogeneity and authenticity of the membrane guanylate cyclase is shown by the following criteria. (i) Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of the purified protein shows a single stained band with a molecular mass of 180 kD (Fig. 1A); (ii) isoelectric focusing of the native and iodinated protein indicates a single symmetrical activity peak with a *p*I of 4.7 ± 0.10 (mean ± SEM) (Fig. 1, B and C); and (iii) Western blot analysis of the GTP affinity-purified enzyme shows a single 180-kD band although the SDS-PAGE of the GTP affinity-purified protein shows multiple Coomassie-stained bands.

The specific activity of the purified particulate guanylate cyclase is 1800 nmol of

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