*Proc. Natl. Acad. Sci. USA*
Vol. 87, pp. 2892–2895, April 1990
Biophysics

# Hormonal regulation of potassium currents in single myometrial cells

(uterus/smooth muscle/norepinephrine/$K^+$ channels/channel modulation)

LIGIA TORO*†, ENRICO STEFANI*†, AND SOLOMON ERULKAR‡

*Department of Physiology, Biophysics and Neurosciences, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, D.F. 07000, México; †Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030; and ‡Department of Pharmacology and Mahoney Institute of Neuroscience, University of Pennsylvania, Philadelphia, PA 19104

*Communicated by Ricardo Miledi, January 29, 1990 (received for review December 19, 1989)*

**ABSTRACT** **Three potassium currents ($I_K$) were recorded from myometrial cells isolated from the uterus of rats at estrus and diestrus and kept in culture for 1–6 days. $I_K$ were differentiated by their modulation with norepinephrine and/or by their onset kinetics. At +50 mV the activation time constants were about 0.7 ms, 6 ms, and 15 ms for the fast, the intermediate, and the slow $I_K$, respectively. Norepinephrine (1 μM) potentiated the fast $I_K$ and reduced the intermediate $I_K$. In addition, differences were found with respect to cells from animals at estrus and diestrus. The fast $I_K$ was preferentially expressed in cultures from animals at estrus, whereas the intermediate $I_K$ was more frequent in cells from rats at diestrus. These results indicate that $K^+$ channels from myometrial cells are multiregulated. Regulation may occur by short-term signals (neurotransmitters) and/or by preferentially expressing distinct types of channels depending on the hormonal status of the animal.**

Uterine smooth muscle contractile activity is regulated by hormones during the estrus cycle, and efforts have been made to correlate the hormonal levels with the mechanical or electrical activity of the muscle (1–4). In addition, myometrium responds to adrenergic stimulation with relaxation or contraction depending on the hormonal state and the species of the animal (4). Experimental evidence supports the idea that $K^+$ channels play a major role in the adrenergic-mediated relaxation or contraction (5–7). β-Adrenergic stimulation activates various types of $K^+$ channels (7, 8), whereas α-adrenergic stimulation inhibits voltage-dependent $K^+$ channels of cardiac myocytes (9).

Potassium currents ($I_K$) with different kinetic properties have been described for the myometrium of pregnant (10) and nonpregnant estradiol-treated rats (11). However, the recorded currents resulted from many cells and no fast clamp could be achieved. Isolated single cells have proven to be useful to study the electrical characteristics of pregnant myometrium (12), where $Ca^{2+}$ and $Na^+$ currents have been described (13, 14). In other smooth muscles, several types of $I_K$ have been demonstrated (15). However, these currents have not been characterized in single cells from nonpregnant myometrium.

We decided to characterize the several $I_K$ in isolated myometrial cells, to analyze their occurrence in estrus and diestrus stages of the estrus cycle, and to evaluate their possible regulation by norepinephrine (NE).

We found three $I_K$: a fast ($I_{K\text{-}f}$), a slow ($I_{K\text{-}s}$), and an intermediate ($I_{K\text{-}i}$). $I_{K\text{-}f}$ was predominant in cells from animals at estrus, whereas $I_{K\text{-}i}$ was more frequent in cells from animals at diestrus. In addition, NE affected the $I_K$ in different ways. These results suggest that the contractile activity of the uterus can be regulated by short-term events such as the actions of neurotransmitters and also by long-term processes such as the action of hormones through a preferential expression of distinct ionic conductances. Preliminary accounts of this work have appeared previously (16).

## METHODS

**Enzymatic Dispersion and Tissue Culture.** Female Wistar rats weighing 150–200 g were used. A vaginal smear was taken to select animals at estrus or diestrus. The isolation and primary culture procedures were similar to those reported previously (17), but the first and second enzyme treatments lasted for 1.5 hr each. Cells were plated onto glass coverslips covered with 100 μg of protamine per ml and kept at 37°C in a humidified atmosphere composed of 95% air/5% $CO_2$. Cells remained differentiated between the first and sixth day of culture and were used within this period.

**Current Monitoring.** Whole cell currents were recorded using a patch-clamp amplifier (L/M-EPC7, List). Patch pipettes were fabricated from borosilicate glass (Boralex 100 μl, Rochester Scientific), siliconized, and fire polished (Narishige microforge, MF-83). Pipette resistances ranged from 1 to 3 MΩ and seal resistances ranged from 10 to 20 GΩ. Data were acquired and analyzed using an IBM AT computer with an analog-digital interface board (Tecmar Labmaster TM100). Linear resistance and capacitive currents were eliminated in all traces after subtracting scaled small depolarizing control pulses (P/4). Control pulses did not elicit nonlinear voltage-dependent currents. In some experiments the electrode had small variations in resistance during whole cell clamp, which modified the charge speed of the combined electrode and membrane capacity. This variation was minimized by giving control pulses before and after each test pulse. Typical values of cell input capacity and electrode resistance were 50 pF and 2 MΩ, respectively, which give a theoretical value of 100 μs for the charge speed of the clamp. However, capacity transients had a somewhat slower time constant of decay of 199 ± 0.09 μs [mean ± SD; $n = 10$ ($n$ = number of cells)], probably due to an additional access resistance. Experiments were carried out at room temperature. Currents were normalized per unit surface assuming the equivalence 1 μF ≡ 1 $cm^2$. Time constants of activation were determined at +50 mV by fitting a single exponential to the current traces. Since at this potential inward currents are minimal they would not introduce significant errors in the measurement.

**Solutions.** Bath solutions were bubbled with $O_2$. Ringer–Krebs solution contained (mM) 145 NaCl, 5 KCl, 1 $MgSO_4$, 5 sodium Hepes, 10 glucose, and 2.5 $CaCl_2$ (pH 7.4). Intra-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: $I_K$, potassium current(s); $I_{K\text{-}f}$, fast potassium current; $I_{K\text{-}i}$, intermediate potassium current; $I_{K\text{-}s}$, slow potassium current; $V_H$, holding potential; NE, norepinephrine.

Table 1. Hormonal regulation of $I_K$ at estrus and diestrus

| Hormonal status | $n$ | $I_{K\text{-}f}$, $\mu A/cm^2$ | $I_{K\text{-}i}$, $\mu A/cm^2$ |
|---|---|---|---|
| Estrus | 18 | 5.2 ± 1 (82) | 4.8 ± 0.8 (66) |
| Diestrus | 15 | 6 ± 4 (13) | 12 ± 2 (93) |

One- to 3-day-old cultured cells were used. Values in parentheses represent the total number (percentage) of cells having each type of current, whether alone or in combination with other currents. Values are the average magnitude of the current per unit surface elicited with a command voltage to +20 mV from a $V_H$ of −90 mV (mean ± SEM; $n$ = number of cells).

cellular $K^+$ solution consisted of (mM) 150 potassium aspartate, 1 $MgCl_2$, 2.5 $CaCl_2$, 10 potassium Hepes, 5 $K_2EGTA$, and 5 $Na_2ATP$ (pH 7.4). All chemicals were from Sigma.

## RESULTS

**$I_K$.** $I_K$ in rat myometrial cells cultured from day 1 through day 6 could be grouped into three different classes on the basis of their onset of activation: $I_{K\text{-}f}$ (fast), $I_{K\text{-}i}$ (intermediate), and $I_{K\text{-}s}$ (slow).

To illustrate the properties of individual $I_K$ we selected cells showing only one of the three currents (Figs. 1–3). This occurred in about half of the total number of cells (42% of 48 cells). The predominance of each current type depends on the hormonal status of the animal (Table 1). Fig. 1 *A* and *B* shows records at different time scales of a fast outward current, labeled $I_{K\text{-}f}$. This current can be characterized by its fast time constant of activation of 0.7 ± 0.3 ms ($n$ = 4), at +50 mV (mean ± SD; $n$ = number of cells). Inactivation of $I_{K\text{-}f}$ did not occur during the 40-ms test pulse, a period of about 60 times the activation time constant of 0.7 ms. When the cell was repolarized to −90 mV ($V_H$) no tail currents were detected. This may be due to the fast kinetics of the tail currents at −90 mV, which would not be detectable in our system. However, tail currents could be measured when the cells were repolarized to more depolarized potentials since they became slower. The resulting tail equilibrium potential was between −80 and −90 mV. Fig. 1*C* shows the normalized current vs. voltage relationship of $I_{K\text{-}f}$ ($n$ = 6; other values are indicated in parentheses). When the cells were depolarized from a $V_H$ of −90 mV to voltages more positive than −50 mV, $I_{K\text{-}f}$ was activated. The maximum slope conductance per unit surface varied from 40 to 200 $\mu S/cm^2$.

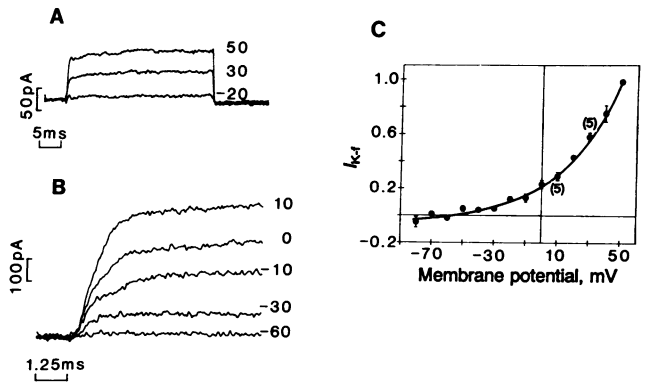


FIG. 1. $I_K$ with fast activation, $I_{K\text{-}f}$. (*A*) Records from a myometrial cell from a rat at estrus on the second day of culture. Traces show the elicited $I_{K\text{-}f}$ from a holding potential ($V_H$) of −90 mV to different command voltages (numbers at the right of each trace). (*B*) Currents from a cell from a rat at estrus on the fourth day of culture. Traces were taken with a faster time base than in *A*, showing depolarizing pulses, from a $V_H$ of −90 mV to values indicated at right of each trace. (*C*) Normalized current vs. membrane potential plot ($n$ = 6, except in those cases indicated on the graph in parentheses). In this and in the following figures bars represent the standard error of the mean.

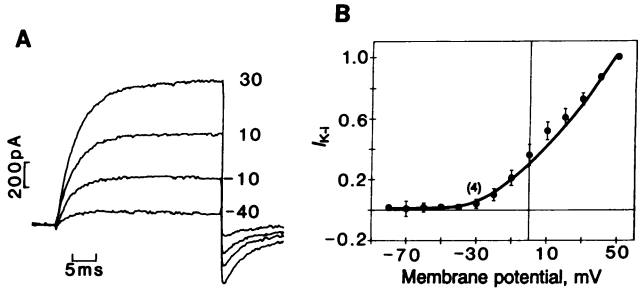


FIG. 2. (*A*) Records of $I_{K\text{-}i}$: Currents from a cell at diestrus on the first day of culture. When the cell was depolarized, from a $V_H$ of −90 mV to potentials more positive than −60 mV (numbers at the right of the traces), $I_{K\text{-}i}$ was elicited. $K^+$ tail currents are seen at the end of the pulse. (*B*) Normalized current vs. membrane potential curve, showing the mean values of five cells (where different, marked in parentheses).

Fig. 2 illustrates a slower outward current, labeled $I_{K\text{-}i}$. Fig. 2 *A* and *B* shows the records and the normalized current vs. voltage relationship, respectively ($n$ = 5; other values are in parentheses). $I_{K\text{-}i}$ had a time constant of activation of 6.0 ± 0.8 ms ($n$ = 8) at +50 mV. The activation level for this current (more positive than −60 mV, Fig. 2*B*) was similar to that for $I_{K\text{-}f}$ and the maximum slope conductance per unit surface varied from 100 to 200 $\mu S/cm^2$.

The main kinetic difference between $I_{K\text{-}i}$ and $I_{K\text{-}f}$ lies in the time constants of their rising phases (6.0 vs. 0.7 ms, respectively). Furthermore, for $I_{K\text{-}i}$ tail currents were recorded upon repolarization to −90 mV (Fig. 2*A*).

A third much slower current, $I_{K\text{-}s}$ (Fig. 3), with a longer time constant of activation (15 ± 1 ms at +50 mV, $n$ = 5) was also found. Fig. 3*A* shows superimposed traces obtained after membrane depolarization with a pulse of 345 ms duration from a holding potential of −90 mV. This current slightly inactivated during longer pulses (3.4 s) (Fig. 3*B*). Fig. 3*C* shows the normalized *I–V* curve from four cells (other $n$ are shown in parentheses). The current was detected when pulses more positive to −30 mV were applied to the patch

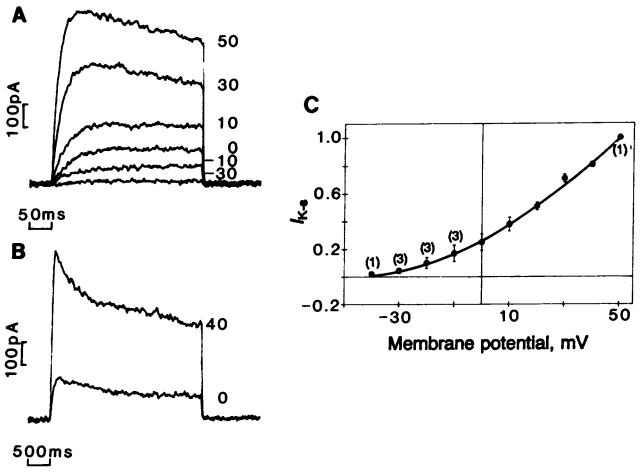


FIG. 3. Slow $I_K$, $I_{K\text{-}s}$. (*A*) Superimposed current traces. $V_H$ = −90 mV. Command voltages are the numbers at the right of each record. The time to reach its maximum value was 68 ± 17 ms ($n$ = 3) when the cells were depolarized to +50 mV. (*B*) $I_{K\text{-}i}$ elicited when longer pulses (3.4 s) were applied, from a holding potential of −90 mV. (*C*) Normalized peak values of $I_{K\text{-}s}$ vs. membrane potential curve. Data are the mean values of four cells (other values are indicated in parentheses).

pipette. Maximum slope conductances per unit surface varied from 160 to 400 $\mu S/cm^2$.

All of these outward currents were absent when internal $K^+$ was replaced by $Cs^+$ and tetraethylammonium ion (TEA) and when the external solution contained TEA and 3,4-diaminopyridine.

**Differential Effect of NE on $I_{K\text{-}i}$ and $I_{K\text{-}f}$.** $I_{K\text{-}i}$ can be differentiated from $I_{K\text{-}f}$ by its response to NE since $I_{K\text{-}f}$ was potentiated and $I_{K\text{-}i}$ was reduced. Fig. 4 *A* and *C* shows records and current vs. voltage relationships of $I_{K\text{-}f}$ before and after the application of 1 $\mu$M NE to the external solution in the vicinity of the cell. The current elicited by a depolarization step from −90 to +50 mV was potentiated with a ratio $G_{NE}/G$ of 4 ± 1 ($n$ = 4), where $G$ = maximum slope conductance and $G_{NE}$ = the maximum slope conductance after addition of NE to the external solution. Fig. 4 *B* and *D* shows that NE had the opposite effect on $I_{K\text{-}i}$. This current was either diminished ($G_{NE}/G$ = 0.6 ± 0.1; $n$ = 4) or abolished ($n$ = 4) by NE. Fig. 4*B* shows the currents obtained before and after the addition of 1 $\mu$M NE, when the cell was pulsed from a $V_H$ of −90 mV to a command voltage of +30 mV. The tail current associated with $I_{K\text{-}i}$ was also reduced by NE.

Fig. 4 shows the effect of NE in cells having one type of $I_K$. A similar distinct effect was observed in cells having a mixture of both $I_K$ (Fig. 5). The application of 1 $\mu$M NE to the bath potentiated $I_{K\text{-}f}$ and simultaneously diminished $I_{K\text{-}i}$. To estimate $I_{K\text{-}i}$, $I_{K\text{-}f}$ was subtracted from the total current from its maximum value (arrows in Fig. 5) attained at about 2 ms after the initiation of the pulse. Note that the tail currents normally associated with $I_{K\text{-}i}$ were also reduced by NE (see also Fig. 4). Finally, in two cells NE was also able to diminish $I_{K\text{-}s}$.

**Currents Are Affected by the Hormonal Status of the Rat.** It is well known that the excitability of the uterus varies according to the hormonal status of the animal (2, 18). We therefore decided to analyze $I_{K\text{-}f}$ and $I_{K\text{-}i}$ in relation to two estrus phases of the rat (estrus vs. diestrus in 1- to 3-day cultures). Interestingly, $I_{K\text{-}i}$ was more frequently recorded in

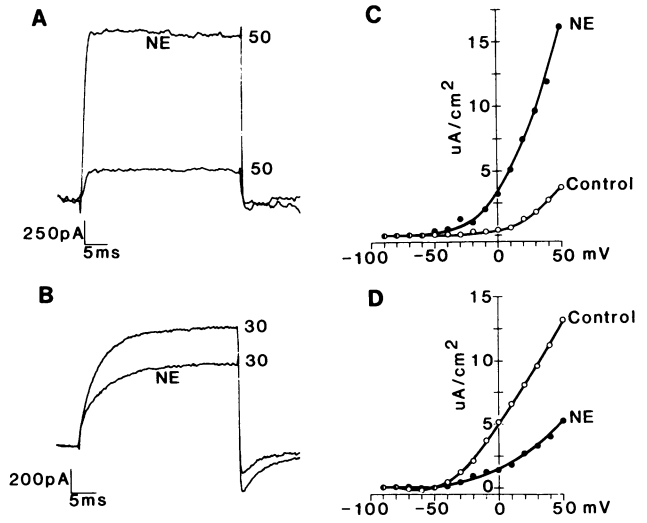


FIG. 4. Opposite effects of NE on $I_{K\text{-}f}$ and $I_{K\text{-}i}$. (*A* and *C*) Three-day culture: Rat in estrus. Traces are the $I_{K\text{-}f}$ observed to a command pulse to +50 mV from a $V_H$ of −90 mV (*A*). Current density vs. voltage curve, before (○) and after (●) addition of 1 $\mu$M NE to the external solution in the vicinity of the cell (*C*). (*B* and *D*) One-day culture: Rat in diestrus. Records and current–voltage relationship, respectively. Traces show $I_{K\text{-}i}$ in response to a command pulse to +30 mV from a $V_H$ of −90 mV before (○) and after (●) the addition of 1 $\mu$M NE to the external solution. Currents during the pulse and tail currents were diminished by NE.

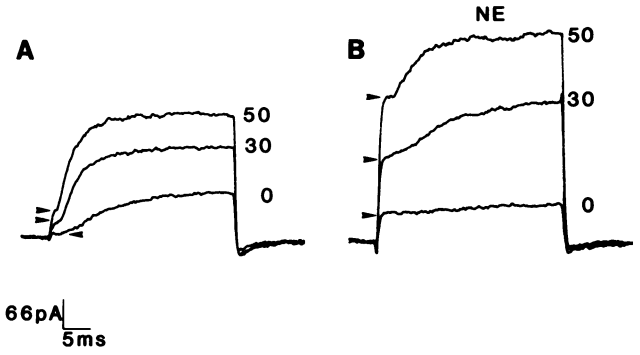


FIG. 5. $I_{K\text{-}f}$ and $I_{K\text{-}i}$ recorded simultaneously from the same cell. Records belong to a cell from a rat at diestrus on the first day of culture. Note that $I_{K\text{-}i}$ superimposes $I_{K\text{-}f}$. To estimate both current amplitudes $I_{K\text{-}f}$ (arrows) was subtracted from the total current. Recording solutions were the same as for Fig. 2. $V_H$ = −90 mV; command voltages are the numbers at the side of the traces. (*A*) Before addition of NE to the external solution. (*B*) After addition of 1 $\mu$M NE to the external solution.

cells that belonged to animals at diestrus opposed to $I_{K\text{-}f}$ that was mainly recorded in cultures from animals at estrus (Table 1).

These results suggest that myometrial cells in culture (1–3 days) are influenced by steroid hormones and possibly maintain their functional properties as in the intact animal.

## DISCUSSION

Single myometrial cells cultured from the nonpregnant uterus of the rat possess three types of $I_K$. Two $I_K$ were differentially regulated by NE. Moreover, the probability that cells express a particular set of channels was dependent on the hormonal status of the animal. This last attribute may play a major role in the contractile activity of the uterus at different stages of the estrus cycle.

$I_K$ have been recorded in strips of estradiol-treated guinea-pig uterus using the sucrose-gap technique (11). This preparation is nearly equivalent to our cells from animals at estrus (estradiol primed) with regard to the hormonal status. Vassort (11) was able to record three outward currents: a "fast" (a maximum reached at 25 ms, at +30 mV), a "slow" (peak amplitude reached after 70–100 ms), and a "late" current (a maximum at 2–3 s). $I_{K\text{-}i}$ (with a maximum at about 27 ms) resembles what he called fast, with respect to its onset time and to the presence of a tail current; $I_{K\text{-}s}$ (with a maximum at about 70 ms) is similar to his slow outward current. However, a current comparable to $I_{K\text{-}f}$ was not reported. This may be due to the experimental conditions used or to basic differences in the species used. It has to be noted though that with the technique used by Vassort (11) the initial part of the traces would be lost due to the capacitive current. This restraint could prevent the author from recording a very fast initial current. In our experiments we have not used long pulses to study the late current (11). This late current has been recorded in *Xenopus* oocytes injected with uterine mRNA from rats treated with $\beta$-estradiol (19).

The physiological relevance of the $I_K$ described in this paper ($I_{K\text{-}f}$, $I_{K\text{-}i}$, and $I_{K\text{-}s}$) can be addressed if we analyze their preferential occurrence during estrus or diestrus in relation to myometrial excitability. During the estrus cycle, the uterus is exposed to different levels of hormones and has different contractile activity (2, 20). Since $I_{K\text{-}f}$ was more frequently encountered in cultures from animals at estrus (Table 1), it is very likely that the physiological role of this type of channel is to prevent spontaneous depolarizations diminishing the excitability of the myometrium at estrus. Accordingly, a similar fast current has also been recorded in vascular smooth

muscle from tail vein of the rat, where only electrotonic responses could be elicited (17).

The fact that $I_{K\text{-}i}$ was more frequently present in cells from animals in diestrus (Table 1) suggests a role for this current in the membrane potential repolarization during the characteristic contractile activity of the rat uterus in this phase (2, 20).

The specific effect of adrenergic agents, such as NE, on myometrium during the estrus cycle is not well established. In this work we have shown that NE had opposite effects on two $I_K$: $I_{K\text{-}f}$ was augmented, whereas $I_{K\text{-}i}$ was diminished. This selective behavior, added to the preferential occurrence of $I_{K\text{-}f}$ (this work) and $\beta$-receptors (21) during estrus, strongly suggest that the major role of NE is to diminish the activity of the uterus at estrus. This effect may take place after the binding of NE to $\beta$-receptors, having as a final step the enhancement of $I_{K\text{-}f}$; this in turn results in hyperpolarization and relaxation (22), although other mechanisms may occur. In contrast, because $I_{K\text{-}i}$ is more frequent at diestrus, the role of NE would be to augment the activity of the uterus at this phase by reducing the hyperpolarizing $I_K$ or by unmasking a $Ca^{2+}$ entry. Along this line we have recently reported that NE can activate or inhibit myometrial $K^+$ channels incorporated into bilayers. The stimulatory effect depends upon the activation of G proteins coupled to $\beta$-adrenergic receptors (23) (L.T., J. Ramos-Franco, and E.S., unpublished data). Opposite responses to NE of two types of $Ca^{2+}$ currents in single vascular smooth muscle cells have also been reported (24). Furthermore, we have also described the existence of $Ca^{2+}$ currents in myometrial cells that were preferentially expressed in diestrus (25).

In conclusion, smooth muscle cells from rat uterus have several types of $I_K$ that are differentially expressed, depending upon the hormonal status of the animal. It is suggested that the expression of distinct types of channels may be mediated by hormones, playing an important role to delineate the specific function of the uterus.

We thank Araceli Navarrete for preparing the cultures and Angel Marín for the analysis programs. This work was supported by grants from the National Institutes of Health (HD25616 to E.S. and MS 12211 to S.E.) and from Consejo Nacional de Ciencia y Tecnología (México) (to E.S.).

1. Marshall, J. M. (1962) *Physiol. Rev.* **42,** 213–227.
2. Batra, S. & Bengtsson, B. (1978) *J. Physiol. (London)* **276,** 329–342.
3. Osa, T. & Maruta, K. (1986) *Jpn. J. Physiol.* **36,** 871–889.
4. Kao, C. Y. (1989) in *Biology of the Uterus*, eds. Wynn, R. M. & Jollie, W. P. (Plenum, New York), pp. 403–453.
5. Kroeger, E. A. & Marshall, J. M. (1973) *Am. J. Physiol.* **225,** 1339–1345.
6. Yamaguchi, H., Honeyman, T. W. & Fay, F. S. (1988) *Am. J. Physiol.* **254,** C423–C431.
7. Kume, H., Takai, A., Tokuno, H. & Tomita, T. (1989) *Nature (London)* **341,** 152–154.
8. Walsh, K. B., Begenisich, T. B. & Kass, R. S. (1989) *J. Gen. Physiol.* **93,** 841–854.
9. Apkon, M. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8756–8760.
10. Mironneau, J. & Savineau, J. P. (1980) *J. Physiol. (London)* **302,** 411–425.
11. Vassort, G. (1975) *J. Physiol. (London)* **252,** 713–734.
12. Mollard, P., Mironneau, J., Amedee, T. & Mironneau, C. (1986) *Am. J. Physiol.* **250,** C47–C54.
13. Amedee, T., Mironneau, C. & Mironneau, J. (1987) *J. Physiol. (London)* **392,** 253–272.
14. Ohya, Y. & Sperelakis, N. (1989) *Am. J. Physiol.* **257,** C408–C412.
15. Tomita, T. (1988) *Jpn. J. Physiol.* **38,** 1–18.
16. Toro, L., Stefani, E. & Erulkar, S. (1987) *Soc. Neurosci. Abstr.* **13,** 1441.
17. Toro, L., Gonzalez-Robles, A. & Stefani, E. (1986) *Am. J. Physiol.* **251,** C763–C773.
18. Kuriyama, H. & Suzuki, H. (1976) *J. Physiol. (London)* **260,** 315–333.
19. Boyle, M. V., Azhderian, E. M., MacLusky, N. J., Naftolin, F. & Kaczmarek, L. K. (1987) *Science* **235,** 1221–1224.
20. Franchi, A. M., Chaud, M., Borda, E. S., Gimeno, M. F., Lazzari, M. A. & Gimeno, A. L. (1981) *Prostaglandins* **22,** 637–649.
21. Krall, J. F., Mori, H., Tuck, M. L., Leshon, S. L. & Korenman, S. G. (1978) *Life Sci.* **23,** 1073–1082.
22. Riemer, R. K. & Roberts, J. M. (1986) in *The Physiology and Biochemistry of the Uterus*, ed. Huszar, G. (CRC, Boca Raton, FL), pp. 53–71.
23. Ramos-Franco, J., Toro, L. & Stefani, E. (1989) *Biophys. J.* **55,** 536 (abstr.).
24. Pacaud, P., Loirand, G., Mironneau, C. & Mironneau, J. (1987) *Pflügers Arch.* **410,** 557–559.
25. Erulkar, S. D., Toro, L. & Stefani, E. (1988) *Puerto Rico Health Sci. J.* **7,** 87 (abstr.).