Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 2927–2930, April 1991
Neurobiology

Single K^+ channels in membrane patches of arterial chemoreceptor cells are modulated by O_2 tension

(O_2 sensing/glomus cells)

MARÍA D. GANFORNINA AND JOSÉ LÓPEZ-BARNEO

Departamento de Fisiología y Biofísica, Facultad de Medicina, Universidad de Sevilla, Avda. Sánchez Pizjuán, 4, 41009 Seville, Spain

Communicated by R. Llinas, December 26, 1990

ABSTRACT **Type I cells of the carotid body are known to participate in the detection of O_2 tension in arterial blood but the primary chemotransduction mechanisms are not well understood. Here we report the existence in excised membrane patches of type I cells of a single K^+ channel type modulated by changes in Po_2. Open probability of the O_2-sensitive K^+ channel reversibly decreased by at least 50% on exposure to hypoxia but single-channel conductance (≈20 pS) was unaltered. In the range between 70 and 150 mmHg (1 mmHg = 133 Pa) the decrease of single-channel open probability was proportional to the Po_2 measured in the vicinity of the membrane patch. The inhibition of K^+ channel activity by low Po_2 was independent of the presence of non-hydrolyzable guanine triphosphate analogues at the internal face of the membrane. The results indicate that the O_2 sensor of type I cells is in the plasma membrane and suggest that environmental O_2 interacts directly with the K^+ channels.**

Type I, or glomus, cells of the carotid body have been considered for decades to be responsible for the detection of oxygen tension (Po_2) in the arterial blood but the mechanisms involved in the process of chemotransduction have remained obscure (1, 2). However, it has been recently discovered that type I cells from adult rabbits can generate action potentials and that they have a voltage-dependent K^+ current selectively and reversibly attenuated by lowering Po_2 (3–8). Inhibition of this K^+ current under hypoxic conditions could produce an increase in the firing frequency of chemoreceptor cells, leading to Ca^{2+} influx, enhanced transmitter release, and activation of the afferent fibers of the sinus nerve (1, 2, 6). The primary site for O_2 detection is, however, unknown. Exposure to cyanide or to extreme hypoxia (<40 mmHg; 1 mmHg = 133 Pa) induces an increase of $[Ca^{2+}]_i$ in type I cells, possibly due to Ca^{2+} release from mitochondria, and a subsequent activation of a Ca^{2+}-dependent K^+ current (9, 10). Therefore it has been argued that the modification of the K^+ current by lowering Po_2 might be a secondary phenomenon rather than an initial step in the process of chemotransduction. Now we report the identification of a single K^+ channel type that fully accounts for the properties of the macroscopic O_2-sensitive K^+ current. Furthermore, we show that in excised membrane patches the activity of these K^+ channels is reversibly modulated by Po_2. Our results support the view that the K^+ channels are directly regulated by O_2 and strongly suggest that the O_2 sensor of chemoreceptor cells is in, or associated with, the plasma membrane. This type of K^+ channel regulation found in the carotid body may have an even broader functional interest because it could also be involved in physiological responses to hypoxia in other tissues.

METHODS

Experiments were performed on enzymatically dispersed type I cells isolated from rabbit carotid bodies. The methods followed in cell dissociation and culture were the same as previously described (3, 4). Cells were plated on fragments of glass coverslips treated with poly(L-lysine). During the experiment a coverslip was transferred to a small chamber of 0.2 ml with continuous flow of solution that could be completely replaced in 10–15 s. Solutions were equilibrated with either air, N_2, or a mixture of both, in order to obtain the desired O_2 tension. Po_2 in the chamber was directly monitored with a polarized 100-μm-thick platinum wire (11) placed in the vicinity of the patch electrode. We used 5- to 8-MΩ patch pipettes fabricated from borosilicate glass. In most experiments cells were first subjected to whole-cell voltage clamp and thereafter the outside-out excised membrane patch configuration was obtained by pulling the electrode away from the cell (12). However, in a few experiments the effect of hypoxia on K^+ channels included in inside-out excised patches was also tested. Composition of solutions and other experimental variables are given in the figure legends.

RESULTS

The major properties of the O_2-sensitive single K^+ channels are summarized in Fig. 1. A macroscopic K^+ current recorded in whole-cell mode during a voltage step to +20 mV is shown in Fig. 1*A*. The current trace, shown for comparison, illustrates the typical time course of the O_2-regulated K^+ current of type I cells, with inactivation during a maintained depolarization (6). After excision of a membrane patch (Fig. 1*B*) the same pulse protocol produced the appearance of single-channel events with a unitary amplitude of 1.8 ± 0.2 pA (mean ± SD, n = 14). The three K^+ channels activated on depolarization open preferentially at the beginning of the pulse and progressively entered an inactivated state. Channel inactivation is clearly evident in Fig. 1*C*, where it is shown the average of 23 consecutive single-channel current sweeps with a time course almost identical to the whole-cell current. Single-channel activity was blocked by 5 mM tetraethylammonium (Fig. 1*D*), which also abolishes the whole-cell current (4). Single K^+ channels similar to those of Fig. 1*B* were found in every experiment and their estimated density is ≈600 per cell. Unitary currents recorded from a patch with at most one open K^+ channel are shown in Fig. 1*E*, which also illustrates the increase in single-channel current amplitude with membrane depolarization, in parallel with the increase of the electrochemical driving force for K^+ movement. Single-channel current amplitude (i) versus membrane potential (V_M) is plotted in Fig. 1*F*. Mean unitary current values from 14 experiments are represented by dots. In these ionic conditions (2.7 mM K^+ in the external solution and 140 mM K^+ in the solution at the internal face of the membrane) the i–V_M relationship was almost linear in the range between −30 and +50 mV, yielding an estimate of the single-channel conductance to be 20.1 pS. In symmetrical 140 mM K^+ (triangles) single-channel current reversed at 0 mV and the unitary conductance was 41.5 pS. These observations indicate that the channels were highly selective for K^+. Ca^{2+}-

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.

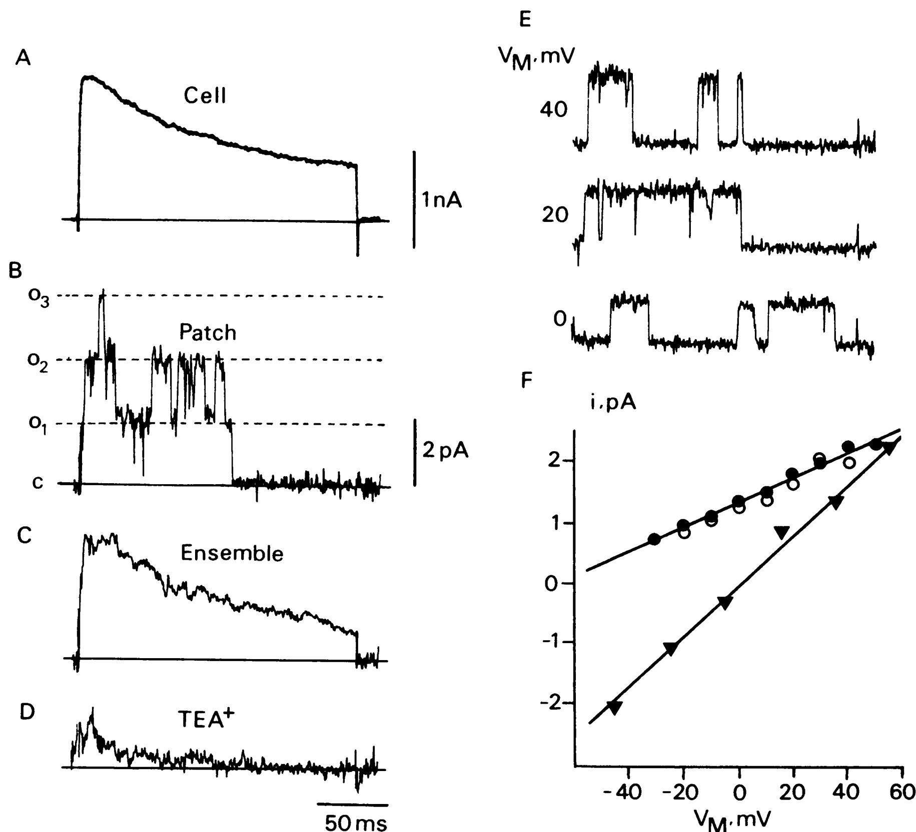

FIG. 1. Properties of the O_2-sensitive K^+ channels. (*A*) Whole-cell K^+ current elicited by a 200-ms pulse from −80 to +20 mV. (*B*) Single-channel events recorded during a similar voltage step in an outside-out excised membrane patch. (*C* and *D*) Average current of 23 consecutive sweeps recorded from the same patch with similar pulse protocol as in *B* (*C*) and blockade of the current by the presence of 5 mM tetraethylammonium (TEA^+) in the external solution (*D*). (*E*) Single-channel current elicited by voltage steps from −80 to 0, +20, and +40 mV in an outside-out patch with at most one open channel. Pulse duration was 200 ms. (*F*) Single-channel current amplitude (*i*) versus membrane potential (V_M). Dots are average currents from 14 patches recorded in asymmetrical K^+ concentrations and with an external solution equilibrated with the normal Po_2 (150 mmHg). The straight line fit has a slope of 20.1 pS. Open circles are average currents from five patches with the same solutions but with low O_2 content (between 80 and 20 mmHg). Triangles are average measurements from two patches in symmetrical K^+ concentrations and normal Po_2. The straight line fit has a slope of 41.5 pS. Current signals were low-pass-filtered at 1 kHz (8-pole Bessel) and digitized with a sampling interval of 500 μs. In all current traces linear ionic and capacity currents were subtracted. Solutions in *A–F* (dots and open circles) contained the following (in mM): external: 140 NaCl, 2.7 KCl, 5 $CaCl_2$, 2 $MgCl_2$, 10^{-3} tetrodotoxin, 10 Hepes; internal: 80 potassium glutamate, 40 KCl, 20 KF, 2 $MgCl_2$, 10 EGTA, 10 Hepes. In the external solution of *D* 5 mM NaCl were replaced by 5 mM tetraethylammonium chloride. In *F* (triangles) the external solution contained the following (in mM): 140 KCl, 5 $CaCl_2$, 2 $MgCl_2$, 10 Hepes. In all solutions pH was between 7.3 and 7.4. Holding potential was −80 mV and temperature was 22–25°C. Current calibration in *B* also applies to traces in *C–E*.

dependent maxi-K^+ channels with unitary conductance of about 250 pS (in symmetrical 140 mM K^+ solutions) were also present in type I cells. Since the activity of maxi-K^+ channels was unaffected by changes in O_2 tension (n = 14), their activation was prevented in most experiments by maintaining [Ca^{2+}] at the internal face of the membrane below 1 nM.

The modulation of the small K^+ channels by Po_2 was investigated in excised membrane patches exposed to solutions with reduced O_2 content. Fig. 2 illustrates the effect of hypoxia on an outside-out excised patch that never showed more than one open channel. Fig. 2*A* shows three sets of current traces recorded during voltage pulses to +20 mV in a control solution equilibrated with air (Po_2 = 150 mmHg), while exposure of the patch to low Po_2 (switching from 150 to 80 mmHg), and after returning to the solution with normal Po_2 (recovery traces). The average of 15–30 consecutive sweeps recorded in the three different experimental conditions are shown in Fig. 2*B*. Open probability of the channel, integrated throughout the pulse duration, in the control solution (p_o = 0.61) decreased markedly during exposure to hypoxia (p_o = 0.28) and returned to a high value (p_o = 0.74) after restoration of normoxic conditions. The recordings clearly show that single-channel current amplitude was unaffected by low Po_2. The average i–V_M values from five patches exposed to hypoxia are plotted in Fig. 1*F* (open circles). The data points indicate that unitary conductance was identical to the control value. Similar qualitative results have been obtained in all patches (either outside-out or inside-out) where the effect of hypoxia was tested (n = 36). Although a detailed study of the effect of hypoxia on the kinetics of the K^+ channel is necessary, our preliminary data suggest that changes in O_2 tension specifically modify activation rate constants. Mean open time (≈18 ms at +20 mV) was the same in control and in hypoxic conditions, whereas mean closed time (20 ms at the same membrane potential and Po_2 = 150 mmHg) increased by at least a factor of 2.5 on exposure to hypoxia. Inactivation time course, evaluated from ensemble average recordings in multichannel patches, seemed to be unchanged by low Po_2.

The reversible decrease of K^+ channel open probability occurs roughly with the time course of Po_2 change in the neighborhood of the membrane patch. Fig. 3*A* shows a continuous electrical signal proportional to the variation of O_2 tension in the chamber. Single-channel events recorded from an outside-out excised patch during 1.3-s voltage steps to +20 mV are shown in Fig. 3*B*. The arrows in Fig. 3*A* indicate the time at which each pulse was delivered. The patch seemed to contain five channels and the number of

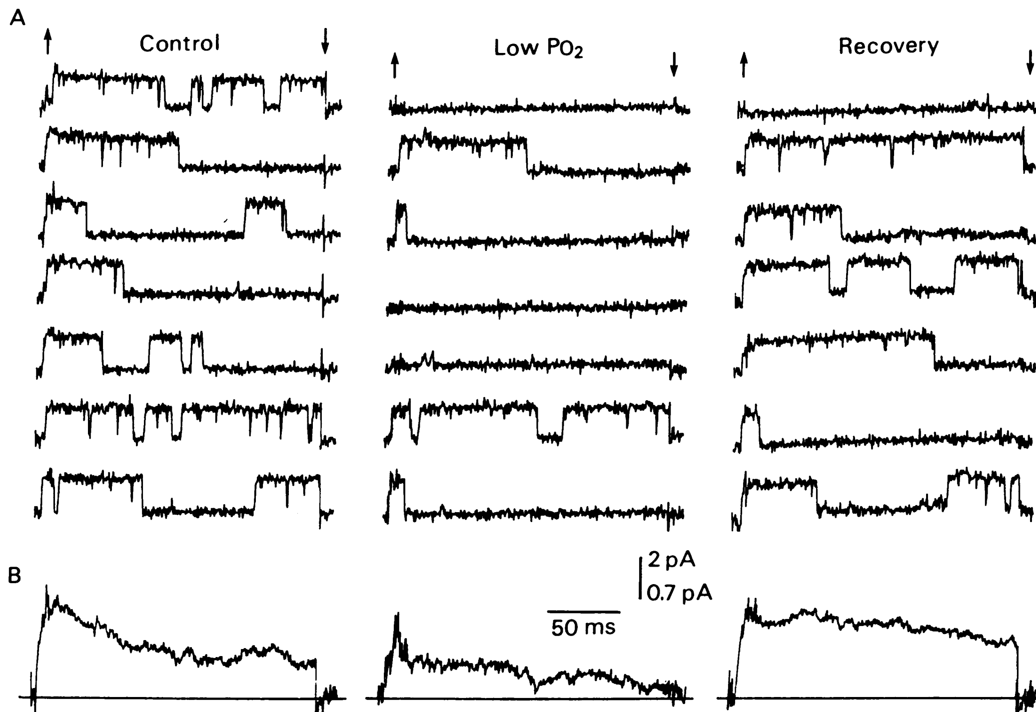

FIG. 2. Modulation of single K^+ channels by O_2 tension. (A) Representative current traces elicited by 200-ms depolarizations from −80 to +20 mV in an outside-out excised membrane patch that contained at most one open channel. The onset and the end of the pulses are indicated by the arrows. Recordings were obtained in the control external solution (equilibrated with air; PO_2 = 150 mmHg), in low PO_2 (switching from 150 to 80 mmHg), and after returning to the solution with normal PO_2. Pulses were applied every 5 s. Current calibration was 2 pA. (B) Ensemble averages of 15–30 consecutive sweeps in the different experimental conditions. Current calibration was 0.7 pA. Open channel probability (control = 0.61; low PO_2 = 0.28; recovery = 0.74) was calculated from the time spent in the open state divided by the duration of the pulses. Solutions and other experimental variables were as in Fig. 1 B–F.

simultaneous events markedly decreased on exposure to hypoxia. The average open probability (p_o), integrated throughout the pulse duration, was 0.34 in normoxic conditions (a; PO_2 = 150 mmHg) but only 0.06 (b), 0.09 (c), and 0.12 (d) with PO_2 values of 85, 44, and 116 mmHg, respectively. Complete recovery of single-channel activity (p_o = 0.25) was obtained on returning to the control solution (e, PO_2 = 145 mmHg). p_o (ordinate) as a function of O_2 tension (abscissa) is plotted in Fig. 3C. Between 70 and 150 mmHg, a range that includes the normal PO_2 values in arterial blood of the rabbit, channel open probability decreased in parallel to O_2 tension. Lowering PO_2 below 70 mmHg produced, however, a relative increase in p_o. These results demonstrate a concentration-dependent effect of O_2 on the K^+ channel.

DISCUSSION

This report shows that a specific kind of K^+ channel of the type I cell plasma membrane is reversibly and selectively modulated by changes in PO_2. Ca^{2+}-dependent maxi-K^+ channels also present in the same preparation are unaffected by alterations in O_2 tension. The O_2-sensitive K^+ channels, which explain the modulation of the macroscopic K^+ current by PO_2 (3), are most likely key elements in the transduction of hypoxic stimuli by type I cells. These K^+ channels are probably involved in the regulation of cell firing and of Ca^{2+} entry through voltage-gated channels during moderate changes in carotid artery PO_2 (2–4). After intoxication of type I cells with cyanide or under extreme hypoxia (with PO_2 values <40 mmHg) secretion may be also supported by strong release of Ca^{2+} from mitochondria (9, 10); however, these conditions are of unlikely physiological occurrence. In fact, we have observed in most experiments a partial recovery of K^+ channel activity at very low PO_2 levels (ref. 6 and Fig. 3 in this report), which may limit the firing frequency of type I cells and contribute to preventing their extensive degranulation.

The inhibition of K^+ channel opening by hypoxia could not be related to the presence of any dialyzable component of the membrane. It was repeatedly observed in a given patch regardless of the time elapsed after excision and was independent of the internal [Ca^{2+}] or the presence of MgATP at the internal face of the membrane. Moreover, the reversible reduction of K^+ channel activity by hypoxia was not altered by the addition of 20 μM GTP[γ-S] (n = 4), which is known to abolish the reversibility of G-protein-mediated modulation of ionic channels (13, 14). These observations suggest that in type I cells O_2 interacts with the K^+ channels either directly or through a site closely associated with them. In this respect type I cells differ from olfactory or taste chemoreceptor cells in which natural stimuli modulate ion channels through the action of intracellular cyclic nucleotides (15–17). In conclusion, our findings demonstrate that environmental O_2 regulates the open probability of a type of K^+ channel in chemoreceptor cells of the carotid body without altering the single-channel conductance. O_2 detection by type I cells seems to involve direct interaction with the K^+ channels, perhaps through a heme-like prosthetic group bound to the channel molecule. This mechanism, without precedent in the literature, may not be restricted to the carotid body but it could also participate in other physiological processes—for example, the autoregulation by local O_2 tension of blood flow in coronary, cerebral, and pulmonary arteries.

We thank Dr. J. Ureña for help in computer programing, Drs. G. Alvarez de Toledo and L. Tabares for comments on the manuscript, and J. R. López-López for his collaboration in the construction of the O_2-sensing microelectrode. Research was supported by a grant from the Dirección General de Investigación Científica y Técnica (PB-86/0250).

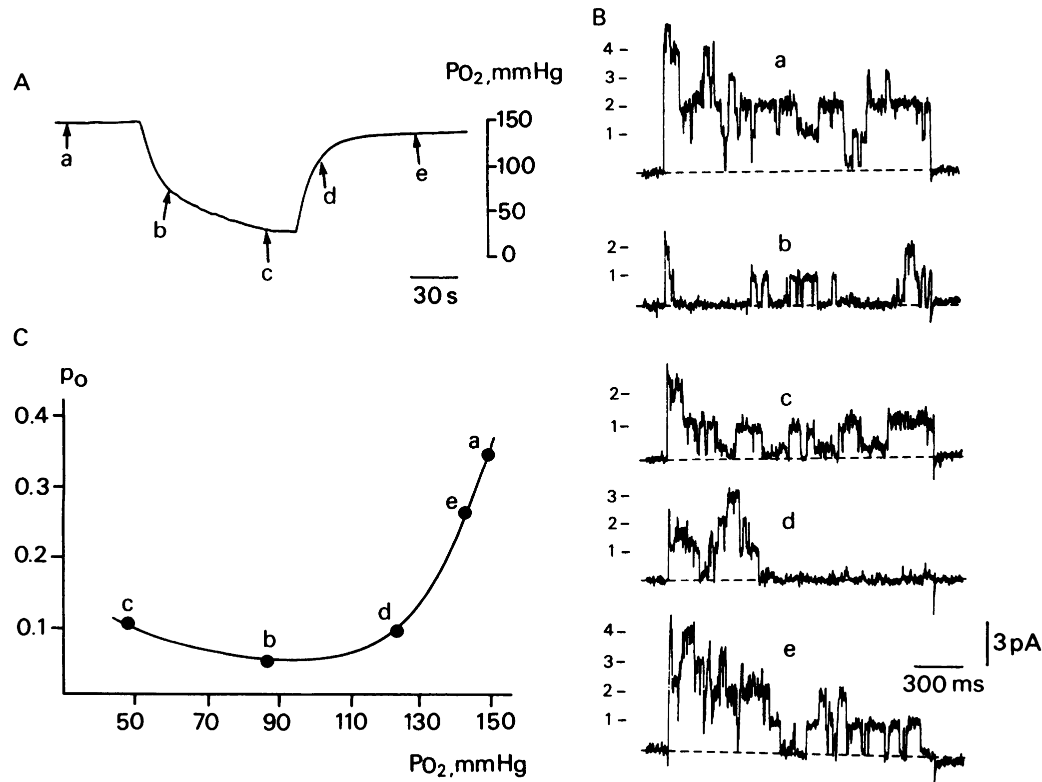

FIG. 3. Changes in O_2 tension and single K^+ channel activity in a multichannel outside-out excised patch. (*A*) Time course of the variations of Po_2 in the recording chamber during a transient exposure to hypoxia. (*B*) Single-channel activity recorded during 1.3-s voltage steps from −80 to +20 mV at the time indicated by the arrows and lowercase letters in *A*. Consecutive pulses were applied every 30 s to allow for complete recovery from inactivation. Average open channel probability (p_o) was calculated from $p_o = (1/Nit) \cdot \int_0^t I dt$, where N = number of channels in the patch, i = single-channel current amplitude, t = pulse duration, and I = net current during the pulse. p_o values were 0.34 (a), 0.06 (b), 0.12 (c), 0.09 (d), and 0.25 (e). (*C*) Channel open probability (p_o, ordinate) as a function of Po_2 (abscissa) in the chamber. Solutions and other experimental conditions were as in Fig. 1 *B–F*.

1. Eyzaguirre, C. & Zapata, P. (1968) in *Arterial Chemoreceptors*, ed. Torrance, R. W. (Blackwell, Oxford), pp. 213–251.
2. Fidone, S. J. & González, C. (1986) in *Handbook of Physiology, The Respiratory System II*, ed. Fishman, A. (Am. Physiol. Soc., Washington), pp. 247–312.
3. López-Barneo, J., López-López, J. R., Ureña, J. & González, C. (1988) *Science* **241**, 580–582.
4. Ureña, J., López-López, J. R., González, C. & López-Barneo, J. (1989) *J. Gen. Physiol.* **93**, 979–999.
5. Duchen, M. R., Caddy, K. W. T., Kirby, G. C., Patterson, D. L., Ponte, J. & Biscoe, T. J. (1988) *Neuroscience* **26**, 291–313.
6. López-López, J. R., González, C., Ureña, J. & López-Barneo, J. (1989) *J. Gen. Physiol.* **93**, 1001–1015.
7. Hescheler, J., Delpiano, M. A., Acker, H. & Pietruschka, F. (1989) *Brain Res.* **486**, 79–88.
8. Delpiano, M. A. & Hescheler, J. (1989) *FEBS Lett.* **249**, 195–198.
9. Biscoe, T. J. & Duchen, M. R. (1989) *J. Physiol.* **416**, 447–468.
10. Biscoe, T. J. & Dunchen, M. R. (1990) *J. Physiol.* **428**, 39–59.
11. Tsacopoulos, M., Poitry, S. & Borsellino, A. (1981) *J. Gen. Physiol.* **77**, 601–628.
12. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. (1981) *Pflügers Arch.* **391**, 85–100.
13. Gilman, A. G. (1987) *Annu. Rev. Biochem.* **56**, 615–649.
14. Brown, A. M. & Birnbaumer, L. (1988) *Am. J. Physiol.* **254**, H401–H410.
15. Nakamura, T. & Gold, G. H. (1987) *Nature (London)* **325**, 442–444.
16. Avenet, C., Hofmann, F. & Lindemann, B. (1988) *Nature (London)* **331**, 351–354.
17. Tonosaki, K. & Funakoshi, M. (1988) *Nature (London)* **331**, 354–356.