Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 4007–4011, May 1990
Chemistry

Continuous monitoring of receptor-mediated changes in the metabolic rates of living cells

(muscarinic, adrenergic, and EGF receptors/transfected cells/glycolysis/microphysiometer/biosensor)

J. C. OWICKI*†, J. W. PARCE*, K. M. KERCSO*, G. B. SIGAL*, V. C. MUIR*, J. C. VENTER‡, C. M. FRASER§, AND H. M. MCCONNELL¶

*Molecular Devices Corp., 4700 Bohannon Drive, Menlo Park, CA 94025; ‡Section of Receptor Biochemistry and Molecular Biology, Laboratory of Molecular and Cellular Neurobiology, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892; §Section on Molecular Neurobiology, Laboratory of Physiologic and Pharmacologic Studies, National Institute on Alcohol Abuse and Alcoholism, Alcohol and Drug Abuse Mental Health Administration, Rockville, MD 20852; ¶Stauffer Laboratory for Physical Chemistry, Stanford University, Stanford, CA 94305

Contributed by Harden M. McConnell, March 12, 1990

ABSTRACT **Activation of β-adrenergic or muscarinic acetylcholine receptors expressed in transfected cells or epidermal growth factor receptors in human keratinocytes produces 15% to 200% changes in cellular metabolic rates. Changes in cell metabolism were monitored continuously with a previously described silicon-based microphysiometer that detects small changes in extracellular pH. The amplitude and kinetics of the metabolic changes depend upon several factors including pretreatment of the cells prior to receptor stimulation, the dose of hormone/neurotransmitter used, and the receptor complement of the cells. Responses are receptor specific; cells transfected with receptor genes respond only to the appropriate hormone/transmitter, whereas control (nontransfected) cells or cells transfected with different receptors exhibit no response. The specificity of the responses was further documented by using pharmacological antagonists. In Chinese hamster ovary (CHO) cells transfected with human β_2-adrenergic receptors, isoproterenol produces a 20–60% increase in the rate of extracellular acidification with an EC_{50} of 4 nM, a response that is competitively antagonized by (−)-propranolol. The EC_{50} for the isoproterenol response is shifted from 4 nM to 100 nM in the presence of 3 nM (−)-propranolol. The kinetics of the metabolic response induced by β-adrenergic receptor stimulation are markedly slower than those elicited by muscarinic receptor agonists. The maximal metabolic response in cells transfected with β-adrenergic receptors peaks at ≈12 min as compared with <30 sec in cells transfected with muscarinic receptors, perhaps reflecting activation of different second-messenger pathways. These findings illustrate an alternative means of studying cellular responses to hormones and neurotransmitters and suggest that metabolic changes will be generally useful for detecting the consequences of receptor–ligand interactions.**

Recent work has shown that the metabolic rates of small samples of living cells can be measured by using a semiconductor-based instrument, the silicon microphysiometer, to detect the rate of excretion of acidic metabolic products (1). These studies revealed the expected qualitative effects of various nonspecific toxic chemicals on the metabolic rates of a variety of cultured cells. In addition, epidermal growth factor (EGF) was shown to affect the metabolic rates of cells containing the EGF receptor. This is not unexpected because increases in cellular glycolytic rates in response to insulin and EGF have been reported and thought to be associated with initiation of a transition of the cells from a dormant (nonproliferating) state to a proliferative state (2).

The ability to measure cellular responses to receptor–ligand interactions on a time scale of minutes suggests that the silicon microphysiometer may be particularly useful for screening new therapeutic drugs. Thus, a major objective of the work reported here was to evaluate this approach as a practical and general method of detecting functional ligand–receptor interactions. To address this question in part, we evaluated changes in cellular metabolic rates elicited by stimulation of receptors that are not normally associated with mitogenesis.

We investigated the effects of two receptors known to mediate their biochemical actions through guanine nucleotide-binding proteins (G proteins), β_2-adrenergic and m_1 muscarinic acetylcholine receptors. These are representative of the major neuroreceptor classes in the sympathetic and parasympathetic nervous systems. Although there are many cell lines available that endogenously contain such receptors, we chose to use Chinese hamster ovary (CHO) cells and murine B-82 cells transfected with the genes encoding each of these receptors. This approach provides a particularly well-defined experimental system and permits the study of the same receptor in different cell types or different receptors in the same cells.

MATERIALS AND METHODS

Materials. Unless otherwise indicated, chemicals were obtained from Sigma and culture media from GIBCO. Transforming growth factor α (TGF-α) was a gift from Rick Harkins (Triton Biosciences, Alameda, CA). EGF was purchased from Clonetics (San Diego), and the anti-EGF antibody was from Collaborative Research.

Keratinocytes. Normal human epidermal keratinocytes were obtained from Clonetics. The cells were cultured in the vendor's keratinocyte growth medium, which is serum-free but contains bovine pituitary extract as well as 10 ng of EGF per ml. The cells were maintained in a humidified atmosphere of 92.5% air/7.5% CO_2, as were all other cells used in the microphysiometer experiments.

Expression of Rat m_1 Muscarinic Acetylcholine Receptors in Murine B-82 Cells. B-82 cells, a murine L cell line, were grown in monolayer culture in medium containing 45% (vol/vol) Ham's F-12 medium, 45% (vol/vol) Dulbecco's modified Eagle's medium, and 10% (vol/vol) denatured fetal bovine serum in a humidified atmosphere of 95% air/5% CO_2. A 2.2-kilobase (kb) *Taq* I–*Bam*HI restriction fragment from genomic clone c71 (3) that contains the entire coding region

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: CHO, Chinese hamster ovary; EGF, epidermal growth factor; PtdIns, phosphatidylinositol; TGF-α, transforming growth factor α; G protein, guanine nucleotide binding protein.

†To whom reprint requests should be addressed.

of a rat m_1 muscarinic receptor was subcloned into the expression vector pMSVneo (4) and used for transfection of B-82 cells as described (3, 5). pMSVneo contains the mouse mammary tumor virus long terminal repeat, which is inducible by glucocorticoids. Expression of the m_1 muscarinic receptor gene was induced by the addition of 1 μM dexamethasone 16–48 hr prior to cell assay. One cell line that expressed $\approx 1 \times 10^5$ copies of the muscarinic receptor per cell was chosen for use in the microphysiometer studies.

Expression of Rat m_1 Muscarinic Receptors Transfected in CHO Cells. CHO-K1 cells (American Type Culture Collection) were grown in a humidified atmosphere of 95% air/5% CO_2 in nutrient F-12 mixture (Ham's) containing 10% fetal bovine serum, 2 mM glutamine, and 50 units of penicillin/50 mg of streptomycin per ml. As previously described, the rat m_1 muscarinic receptor has been expressed in CHO cells, and its pharmacology and biochemistry have been characterized in detail (6). One cell line expressing approximately 2×10^5 muscarinic receptors per cell was used for microphysiometer studies.

Expression of Human β_2-Adrenergic Receptors in CHO Cells. A 1.2-kb *Nar* I–*Dra* I fragment of human genomic clone CLFV-108 (7) was cloned into the *Sma* I–*Acc* I sites of pGEM-3Z to obtain new restriction sites at the ends of the DNA fragment. This construct was digested with *Sph* I, blunt-ended with Klenow DNA polymerase, and digested with *Sac* I, liberating a 1.2-kb fragment of DNA that was cloned into the *Sma* I–*Sac* I sites of the expression vector, pSVL. CHO cells were cotransfected with pSVL and pMSVneo as described (6). For some of the studies described, a cell line containing $\approx 1.2 \times 10^6$ β_2-adrenergic receptors per cell was used.

Expression of Human β_2-Adrenergic Receptors in B-82 Cells. A B-82 cell line expressing human β_2-adrenergic receptors at a density of 900 fmol/mg of membrane protein ($\approx 1.2 \times 10^5$ receptors per cell) has been described (8). These cells were used for some of the microphysiometer studies described in this work.

Cell Culture. Cells to be used in the microphysiometer were plated onto coverslips in plastic Petri dishes at the time of subculture and then allowed to grow to 60–90% confluence. Serum starvation was found to potentiate the metabolic responses. Therefore, 12–28 hr before the coverslips were to be used, the medium was removed from the Petri dishes. The dishes were washed twice with 10 ml and then refilled with 15 ml of serum-free culture medium. EGF and bovine pituitary extract were similarly withheld from the medium used with keratinocytes. For cells transfected with the muscarinic receptor gene in pMSVneo, the Petri dishes were refilled with serum-free medium containing 1 μM dexamethasone.

Measurement of Cellular Metabolic Rates. Cellular metabolic rates were measured with the silicon microphysiometer as has been described elsewhere in detail (1). Briefly, the microphysiometer contains a microvolume flow chamber in which cells are immobilized. One wall of the chamber is a silicon-based light-addressable potentiometric sensor (LAPS; ref. 9) that can measure small changes in solution pH. Apposed to and about 100 μm distant from the sensor is a coverslip bearing the adherent cells to be tested.

The medium that is pumped through the channel between sensor and coverslip differs from normal growth medium by the absence of bicarbonate. This reduces buffer capacity and enhances pH changes. In the experiments described here, serum was also excluded.

For a measurement of metabolic rate, the flow of medium is temporarily halted. The cells then acidify the medium in the chamber because of the production of lactate and, to a lesser extent, CO_2. When flow is resumed after a stagnant period of 30–200 sec, the pH in the chamber rises (typically 0.1–0.2 pH units), returning to that of the fresh medium. The metabolic rate of the cells is taken as the rate of chamber acidification while flow is off, generally determined as the slope of a linear least-squares fit to the pH-vs.-time data. This process is repeated for every measurement of metabolic rate. To determine the effect of various compounds on the metabolic rate of the cells, acidification rates are measured on the same population of cells before and after introduction of the compound into the flow chamber.

RESULTS

As previously demonstrated, application of EGF to resting keratinocytes increases their metabolic rate (1). This stimulation peaks $\approx$15 min after exposure to EGF and is specific for EGF, as shown by inhibition upon simultaneous addition of anti-EGF antibody (Fig. 1*A*). The closely related mitogen TGF-α also causes a metabolic change indistinguishable from that of EGF (Fig. 1*B*).

Infusion of the muscarinic receptor agonist carbamylcholine (carbachol, 100 μM) into the chamber containing CHO cells expressing rat m_1 muscarinic receptors results in a rapid rise in cellular metabolic rate (Fig. 2). The increase in metabolic rate peaks at $\approx$180% of the unstimulated rate, and this peak occurs at or before the first data point collected subsequent to the addition of the agonist ($\approx$100 sec). This response is completely blocked in the presence of the muscarinic antagonist atropine (1 μM), indicating that the response is mediated through the muscarinic receptor in these transfected cells.

To better resolve the kinetics of the change in metabolic rate elicited by muscarinic receptor stimulation in transfected CHO cells, the acidification data (pH vs. time when the flow is off) were subjected to continuous derivative analysis (10) as opposed to a least-squares fit to a straight line. With derivative analysis, changes in metabolic rates can be measured on the same time scale as single pH measurements ($\approx$1–10 sec). In these transfected CHO cells, the metabolic rate is highest at the first data point collected after addition of carbachol (Fig. 3). We estimate this first point to represent the metabolic rate within 30 sec of carbachol reaching the cells.

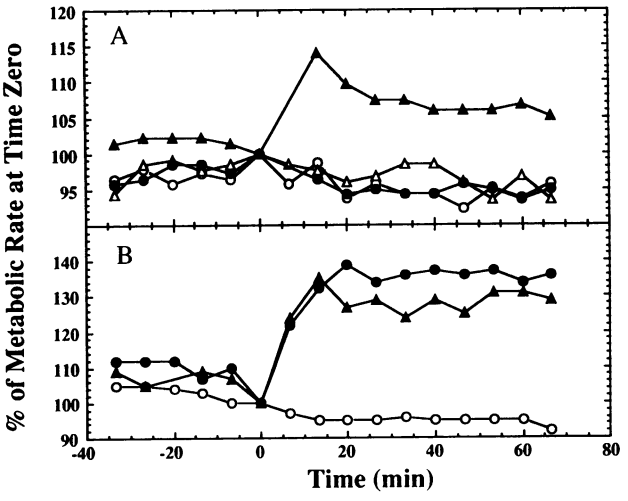

FIG. 1. Metabolic response of keratinocytes to growth factors. (*A*) Four chambers containing human keratinocytes were operated in parallel. At time $t = 0$, medium containing EGF at 5 ng/ml (▲), EGF at 5 ng/ml and anti-EGF antibody at 12.5 μg/ml (●), anti-EGF antibody at 12.5 μg/ml (△), or no additives (○) was introduced separately into the flow streams of the four chambers. (*B*) Three chambers containing human keratinocytes were operated in parallel. At time $t = 0$, medium (○) or medium supplemented with EGF at 10 ng/ml (▲) or with TGF-α at 10 ng/ml (●) was added separately to one of the three chambers containing normal human keratinocytes. Cellular metabolic rates were measured as described. Data are taken from a representative experiment.

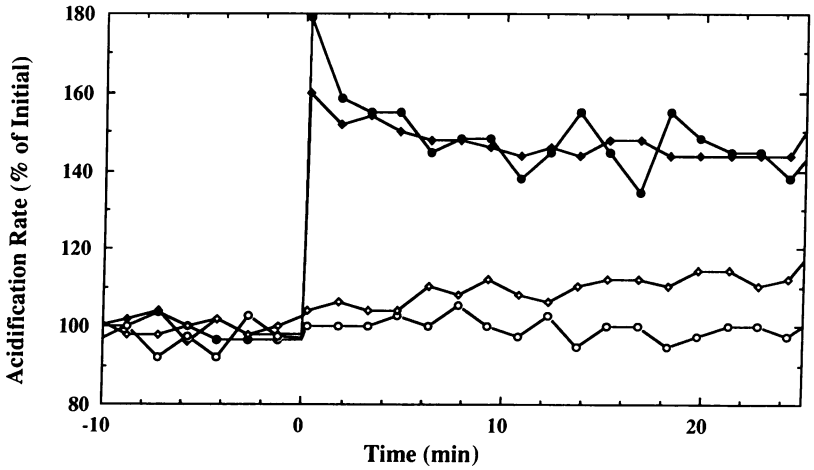

FIG. 2. Metabolic responses of CHO cells transfected with the muscarinic acetylcholine receptor to agonist and antagonist. Cells in four microphysiometer chambers were exposed either to control medium (●, ◆) or control medium containing 1 μM atropine (○, ◇), a muscarinic antagonist. The flow of medium alternated between 30 sec on and 60 sec off. At t = 0, 100 μM carbachol was introduced into all four chambers. Atropine abrogated the response to carbachol. Note that the carbachol-induced increase in metabolic rate was present at the first datum point after carbachol addition. See Fig. 3 for a more detailed analysis of the period around t = 0. Data are taken from a representative experiment.

In order to investigate whether the response observed following the activation of muscarinic receptors can be generalized to other receptors, changes in cellular metabolism were studied in CHO cells transfected with another G protein-linked receptor, the human β_2-adrenergic receptor. Isoproterenol elicits a similar peak metabolic response (120–160% of the rate in unstimulated cells) to that observed with carbachol and the muscarinic acetylcholine receptor (Fig. 4). However, the time course of β_2-adrenergic receptor-induced changes in cellular metabolism is markedly slower than that observed with the muscarinic receptor. The maximal response of the transfected cells to isoproterenol peaks at ≈12 min after the first exposure to agonist. The isoproterenol response is reversible, as removal of isoproterenol from the microphysiometer chamber or addition of propranolol results in an immediate attenuation of the metabolic rate ($t_{1/2}$ = 6 min). This rapid reversal demonstrates that the cellular response requires the continual presence of isoproterenol.

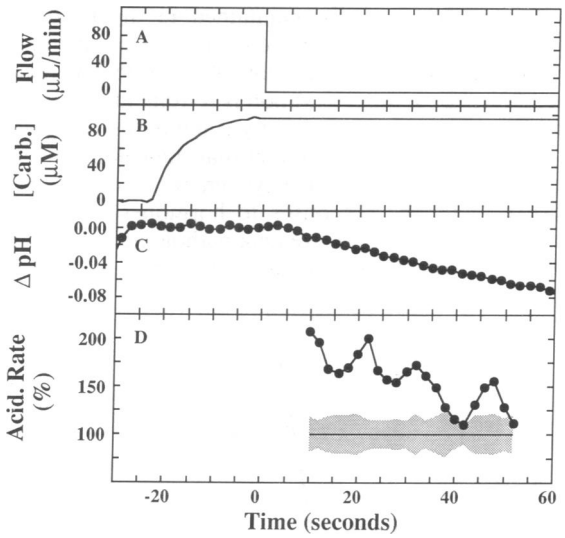

FIG. 3. Kinetic resolution of the early metabolic response to agonist stimulation of the m_1 muscarinic receptor. This figure is a more detailed analysis of the period near t = 0 in Fig. 2. (*A*) Flow rate. At t = −30 sec, carbachol was introduced into the tubing leading to the chamber. Flow was halted at t = 0 sec. (*B*) Carbachol concentration ([Carb.]) in the flow chamber: time course of the arrival of carbachol in the flow chamber. The interface was broadened by parabolic flow between the valve and chamber, and the carbachol nearly reached its full concentration of 100 μM by the cessation of flow at t = 0. Data were obtained by observing the kinetics of pH changes at the sensor when streams of buffer with different pH values were switched in the same apparatus. (*C*) pH in the flow chamber. The pH in the chamber was steady while the flow was on and decreased when the flow stopped. The first data point reflects the end of recovery from the previous flow-off period. pH was taken relative to that of fresh culture medium (7.3–7.4). (*D*) Acidification (Acid.) rates. Point-by-point time derivatives of the pH in the chamber during the measurement period, computed as described in the text. The line at 100% represents the average acidification rate for the five flow-off periods prior to the introduction of carbachol; the stippled area is a 1-standard-deviation envelope of these control data. The carbachol-induced increase in metabolic rate was present at the first data point.

To determine whether the increase in metabolic rate of the transfected CHO cells showed a graded response to agonist concentration, cumulative dose–response experiments were performed. Isoproterenol produced a dose-dependent change in acidification rate with an EC_{50} of 4 nM (Fig. 5). Increasing concentrations of the antagonist, propranolol caused a parallel shift to the right in the dose–response curve for isoproterenol, indicating that the isoproterenol-mediated changes in cellular metabolic rate are competitively antagonized by propranolol.

To study the extent to which muscarinic- and β-adrenergic-mediated metabolic stimulation depends on cell type, similar experiments were performed with B-82 cells transfected with the same two receptors. The time course and amplitude of the metabolic response to 100 μM carbachol of B-82 cells expressing the muscarinic receptor were similar to those of transfected CHO cells (Fig. 6*A*). Likewise, addition of 5 μM isoproterenol to B-82 cells containing β-adrenergic receptors produced an increase in metabolic rate with kinetics and amplitude comparable to those observed with the receptor expressed in CHO cells (Fig. 6*B*). Simultaneous addition of propranolol completely abrogated the cellular response to the agonist (data not shown).

DISCUSSION

In the present study we show that an increase in cellular metabolic rate is a general response of a cell to hormonal stimulation. This response has been demonstrated in three different cell types with receptors that use at least three different second-messenger systems. The cell types used include normal human keratinocytes, CHO cells, and murine fibroblasts (B-82 cells). The EGF receptor is a tyrosine kinase capable of autophosphorylation as well as phosphorylation of other cellular proteins (11), and it has also been shown to activate phosphatidylinositol (PtdIns) hydrolysis (12); the m_1 muscarinic receptor stimulates PtdIns hydrolysis, arachidonic acid release, cAMP accumulation, and changes in K^+ conductance (5, 6, 13, 14); the β_2-adrenergic receptor activates adenylate cyclase (8).

Increases in cellular glycolytic rates in response to insulin and EGF have been documented in several different cell types (2). Although these hormones are mitogenic, the in-

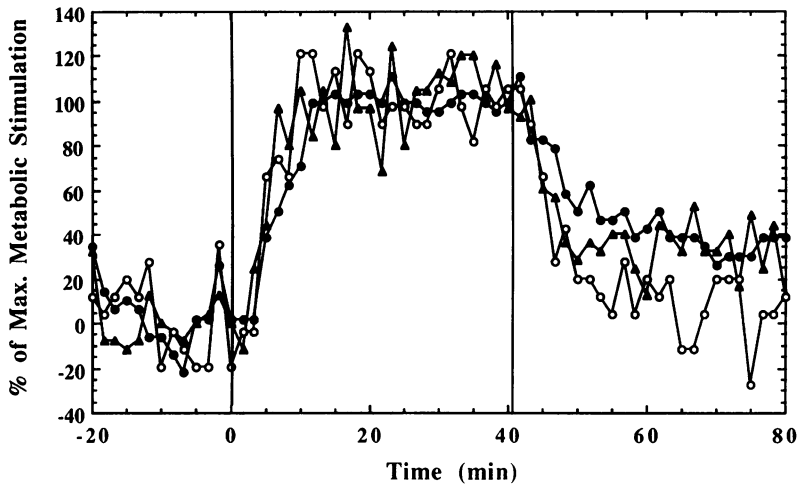

FIG. 4. Kinetics of the metabolic response of CHO cells transfected with the β_2-adrenergic receptor. Three chambers containing CHO cells expressing β_2-adrenergic receptors were perfused with medium. The flow-cycle protocol was 50 sec on and 50 sec off. Medium containing 10 nM isoproterenol was added to all three chambers at $t = 0$ (first vertical line). After metabolic rates stabilized, the medium was changed (second vertical line) to that containing no additions (○), 100 nM propranolol (▲), or 10 nM isoproterenol and 100 nM propranolol (●). Each curve illustrates data from a single representative experiment.

crease in glycolysis proceeds in the presence of inhibitors of protein synthesis (15). These glycolytic increases have been shown to correlate with increases in the intracellular concentration of the phosphofructokinase (PFK-1) stimulator, fructose 2,6-bisphosphate (16). Extracellular calcium has been shown to be required for maximal stimulation of glycolysis in response to these hormones. All of these studies focused on the premise that the increase in glycolysis is associated with initiation of a transition of the cells from a dormant (nonproliferating) state to a proliferative state. In this report we demonstrate increases in cellular metabolic rates as a consequence of agonist binding to two receptors that are not normally associated with mitogenesis.

Although a few reports have appeared suggesting that G protein-linked receptors may influence cell growth, the data from these studies vary depending on cell and receptor types (13, 17, 18). Thus, it is not yet clear whether the metabolic responses initiated by G protein-linked receptors reflect changes in the proliferative state of the transfected cells that are similar to those elicited by EGF.

The metabolic responses to receptor stimulation are best observed in cells that have been maintained in medium free of serum for a period of time prior to the experiment. These "serum-starved" cells give robust responses to receptor stimulation, whereas cells maintained in the presence of serum show reduced or, in some cases, no increase in metabolic rate, upon introduction of the relevant receptor ligand. Although serum starvation is routine in bioassays for growth factors, it is not clear why it improves activation of cells via neuroreceptors. The fact that the cells show stable metabolic rates in the microphysiometer and demonstrate reversible metabolic responses to hormones suggests that serum starvation does not create a pathologic condition that results in physiologically irrelevant responses.

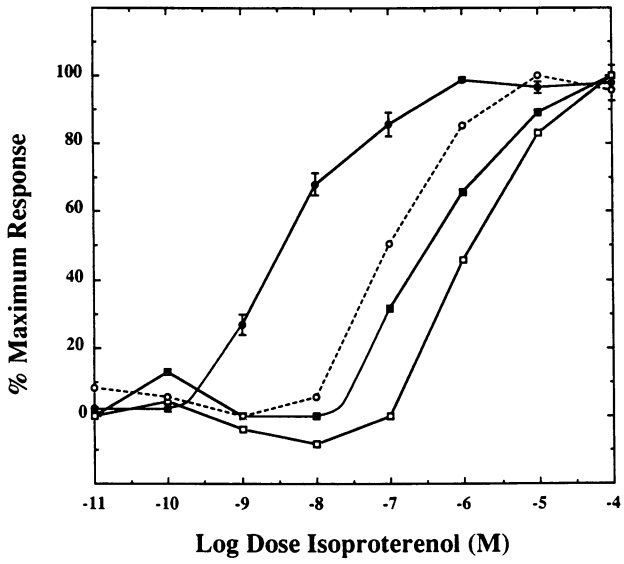

FIG. 5. Cumulative dose–response curves for the response of transfected CHO cells to isoproterenol. The chambers were infused with medium (●) or with medium containing 3 nM (○), 30 nM (■), or 100 nM (□) propranolol. Beginning at 10 pM, isoproterenol was sequentially added to each chamber, with a 10-fold increase in concentration at each step. After each application of agonist, the metabolic rate was allowed to stabilize (≈30 min) before the next higher concentration was added. The metabolic stimulation is normalized and scaled for each curve so that 0% represents the metabolic rate before the addition of isoproterenol and 100% represents the highest observed rate (elicited by 10 or 100 mM isoproterenol). The metabolic rate at 100% stimulation represents an ≈1.5-fold increase in metabolic rate. The error bars represent the SEM for seven determinations from six different experiments carried out over a period of 16 days.

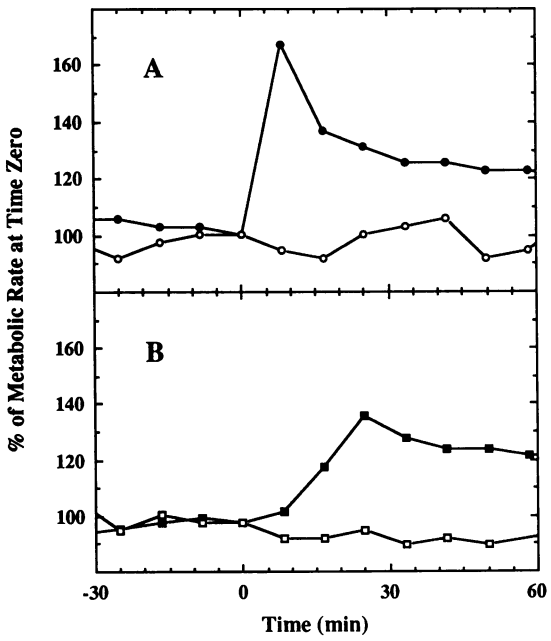

FIG. 6. Agonist stimulation of cellular metabolism in transfected B-82 cells. (*A*) ●, Exposure to 100 μM carbachol beginning at $t = 0$ of B-82 cells expressing the m_1 muscarinic receptor; ○, a parallel sham exposure. (*B*) ■, Exposure to 5 μM isoproterenol beginning at $t = 0$ of B-82 cells expressing the human β_2-adrenergic receptor; □, a parallel sham exposure. Data illustrate representative experiments.

The magnitudes of the observed receptor-mediated increases in metabolic rate range from 15% to 200% of the prestimulation value. This amplitude depends on the treatment of the cells prior to the experiment (e.g., serum starvation). It also depends on the concentration of ligand introduced into the microphysiometer, which makes possible the generation of dose–response curves for assessing the potency of agonists and antagonists. The isoproterenol concentration sufficient to produce half-maximal metabolic stimulation in transfected CHO cells is 4 nM, which is similar to the value previously reported for half-maximal increases in adenylate cyclase activity induced by isoproterenol in a similar transfected system (8).

The large increases in metabolic rate that are observed presumably are due to increases in glycolysis and aerobic metabolism required to make up for an energy deficit created by receptor activation. A quantitative accounting of the cellular energy consumption due to receptor triggering is difficult to estimate from metabolic pathway data since fluxes through second-messenger pathways are not typically measured. The bulk of the energy consumed by resting cells apparently serves to maintain ion gradients across membranes (19, 20). Since receptor activation produces such a large fractional increase in cellular energy demand, it is likely that this increase is required to reestablish ion gradients that have been partially dissipated either directly by the receptor or indirectly by a second-messenger system.

Energy consumption by the cells can be estimated as follows. Typical resting cells produce $\approx 10^8$ protons per cell per sec (1). This corresponds to the turnover of at least 10^8 ATP molecules per cell per sec, based on the catabolism of glucose by glycolysis (1 ATP/H^+) and aerobic respiration (6 ATP/H^+). Measurements of cAMP levels in β-adrenergic-transfected B-82 cells upon stimulation with isoproterenol (8) indicate that about 5×10^4 cAMP molecules are produced per cell per sec. The rate of ATP hydrolysis to form cAMP alone clearly falls orders of magnitude short of explaining the energy demand required to produce the metabolic increases observed in these cells. If the metabolic enhancements produced by β-adrenergic receptors are absolutely dependent on cAMP production, then tertiary biochemical events such as those mediated by protein kinases must be involved. This hypothesis is consistent with the slow time course of metabolic changes observed in cells expressing β-adrenergic receptors.

Whole-cell patch-clamp experiments on A9 L cells transfected with the m_1 muscarinic receptor have demonstrated K^+ currents of 140 pA in response to acetylcholine administration (14), equivalent to $\approx 8 \times 10^8$ K^+ per cell per sec. Assuming the Na^+/K^+-ATPase is used to pump the K^+ back into the cell, then $\approx 4 \times 10^8$ ATP molecules per sec would be required to maintain homeostasis. This value is in good agreement with the observed increases in the rate of proton production following muscarinic receptor activation in the transfected cells. The onset of K^+ currents through the plasma membrane begins ≈ 3 sec after addition of acetylcholine, consistent with the rapid onset of metabolic changes measured with the microphysiometer. The above arguments are consistent with the hypothesis that the metabolic responses to receptor stimulation are largely due to triggered ion fluxes within the cell. This further suggests that it should be possible to use the microphysiometer to measure triggering of ion channel receptors such as nicotinic acetylcholine and γ-aminobutyric acid (GABA) receptors.

Although EGF and muscarinic receptors both produce increases in PtdIns hydrolysis, the kinetics of the metabolic changes induced by these receptors are markedly different. These data would suggest that breakdown of PtdIns may be unrelated to the increase in cellular metabolic rates. However, it is clear from recent studies that G protein-linked receptors are capable of stimulating multiple intracellular effector systems (13, 21, 22), suggesting that the metabolic change observed with the microphysiometer may reflect the activation of several independent pathways.

The kinetics of the metabolic response for a given receptor appear to be related more to the identity of the receptor than to the cell type. It is not yet clear whether a correlation exists between the kinetics of the metabolic response and the second-messenger system activated. It is interesting that the kinetics of the response of B-82 cells to prostaglandin E_1 (PGE_1; ref. 23) is very similar to that for the β-adrenergic response in transfected B-82 cells. PGE_1 and isoproterenol produce nearly identical increases in adenylate cyclase activity in transfected B-82 cells (8). However, the different kinetics of metabolic stimulation by EGF and the muscarinic receptor noted above underline the complexity of cytoplasmic events following receptor stimulation.

Since metabolic responses have now been observed in three different cell types with receptors that use at least three different second-messenger systems, it is likely that measurable changes in metabolic rates occur upon receptor stimulation for a wide variety of receptors. The finding that the kinetics of the metabolic response may vary as a function of the receptor type may allow the use of a single cell expressing multiple receptors for the differential screening for several ligands simultaneously.

We gratefully acknowledge support from the Defense Advanced Research Projects Agency and the U.S. Army Chemical Research and Development Engineering Command, Army Research Office Contract DAAL03-86-C-0009.

1. Parce, J. W., Owicki, J. C., Kercso, K. M., Sigal, G. B., Wada, H. G., Muir, V. C., Bousse, L. J., Ross, K. L., Sikic, B. I. & McConnell, H. M. (1989) *Science* **246**, 243–247.
2. Diamond, I., Legg, A., Schneider, J. A. & Rozengurt, E. (1978) *J. Biol. Chem.* **253**, 866–871.
3. Lai, J., Mei, L., Roeske, W. R., Chung, F.-Z., Yamamura, H. I. & Venter, J. C. (1988) *Life Sci.* **42**, 2489–2502.
4. Chung, F.-Z., Wang, C.-D., Potter, P. C., Venter, J. C. & Fraser, C. M. (1988) *J. Biol. Chem.* **263**, 4052–4055.
5. Mei, L., Lai, J., Roeske, W. R., Fraser, C. M., Venter, J. C. & Yamamura, H. I. (1989) *J. Pharmacol. Exp. Ther.* **248**, 661–670.
6. Fraser, C. M., Wang, C.-D., Robinson, D. A., Giocayne, J. D. & Venter, J. C. (1989) *Mol. Pharmacol.* **36**, 840–847.
7. Chung, F.-Z., Lentes, K. U., Giocayne, J. D., Fitzgerald, M. G., Robinson, D. A., Kerlavage, A. R., Fraser, C. M. & Venter, J. C. (1987) *FEBS Lett.* **211**, 200–206.
8. Fraser, C. M., Chung, F.-Z. & Venter, J. C. (1987) *J. Biol. Chem.* **262**, 14843–14846.
9. Hafeman, D. G., Parce, J. W. & McConnell, H. M. (1988) *Science* **240**, 1182–1185.
10. Savitsky, A. & Golay, M. J. E. (1964) *Anal. Chem.* **36**, 1626–1639.
11. Schlessinger, J. (1986) *J. Cell Biol.* **103**, 2067–2072.
12. Moolenaar, W. H., Bierman, A. J., Tillie, B. C., Verlaan, I., Defize, L. H. K., Honegger, A. M., Ulrich, A. & Schlessinger, J. (1988) *EMBO J.* **7**, 707–710.
13. Conklin, B. R., Brann, M. R., Buckley, N. J., Ma, A. L., Bonner, T. I. & Axelrod, J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8698–8702.
14. Jones, S. V. P., Barker, J. L., Bonner, T. I., Buckley, N. J. & Brann, M. R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4056–4060.
15. Schneider, J. A., Diamond, I. & Rozengurt, E. (1978) *J. Biol. Chem.* **253**, 872–877.
16. Farnararo, M., Vasta, V., Bruni, P. & D'Alessandro, A. (1984) *FEBS Lett.* **171**, 117–120.
17. Ashkenazi, A., Ramachandran, J. & Capon, D. J. (1989) *Nature (London)* **340**, 146–150.
18. Hen, R., Axel, R. & Obici, S. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 4785–4788.
19. Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. & Watson, J. D. (1989) *Molecular Biology of the Cell* (Garland, New York), 2nd Ed., p. 304.
20. Harris, S. I., Balaban, R. S., Barrett, L. & Mandel, L. J. (1981) *J. Biol. Chem.* **256**, 10319–10328.
21. Fraser, C. M., Arakawa, S., McCombie, W. R. & Venter, J. C. (1989) *J. Biol. Chem.* **264**, 11754–11761.
22. Cotecchia, S., Kobilka, B. K., Daniel, K. W., Nolan, R. D., Lapetina, E. Y., Caron, M. G., Lefkowitz, R. J. & Regan, J. W. (1990) *J. Biol. Chem.* **265**, 63–69.
23. Parce, J. W., Owicki, J. C. & Kercso, K. M. (1990) *Ann. Biol. Clin.*, in press.