

# Membrane Responses to Norepinephrine in Cultured Brown Fat Cells

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**ABSTRACT** We used the "perforated-patch" technique (Horn, R., and A. Marty, 1988. *Journal of General Physiology*. 92:145–159) to examine the effects of adrenergic agonists on the membrane potentials and membrane currents in isolated cultured brown fat cells from neonatal rats. In contrast to our previous results using traditional whole-cell patch clamp, 1–23-d cultured brown fat cells clamped with the perforated patch consistently showed vigorous membrane responses to both  $\alpha$ - and  $\beta$ -adrenergic agonists, suggesting that cytoplasmic components essential for the thermogenic response are lost in whole-cell experiments. The membrane responses to adrenergic stimulation varied from cell to cell but were consistent for a given cell. Responses to bath-applied norepinephrine in voltage-clamped cells had three possible components: (a) a fast transient inward current, (b) a slower outward current carried by  $K^+$  that often oscillated in amplitude, and (c) a sustained inward current largely by  $Na^+$ . The fast inward and outward currents were activated by  $\alpha$ -adrenergic agonists while the slow inward current was mediated by  $\beta$ -adrenergic agonists. Oscillating outward currents were the most frequently seen response to norepinephrine stimulation. Activation of this current, termed  $I_{K,NE}$ , was independent of voltage and seemed to be carried by  $Ca^{2+}$ -activated K channels since the current oscillated in amplitude at constant membrane potential and gradually decreased when the cells were bathed with calcium-free external solution.  $I_{K,NE}$  had a novel pharmacology in that it could be blocked by 4-aminopyridine, tetraethylammonium, apamin, and charybdotoxin. Both  $I_{K,NE}$  and the voltage-gated K channels also present in brown fat (Lucero, M. T., and P. A. Pappone, 1989a. *Journal of General Physiology*. 93:451–472) may play a role in maintaining cellular homeostasis in the face of the high metabolic activity involved in thermogenesis.

## INTRODUCTION

Brown adipose tissue responds both metabolically, with large increases in heat production, and electrically, with a triphasic change in membrane potential, when stimulated by norepinephrine (for recent reviews see Nedergaard and Lindberg, 1982; Nicholls and Locke, 1984; Horwitz, 1989). The bulk of the metabolic responses

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involves activation of  $\beta$ -adrenergic receptors, but there is also a small contribution from  $\alpha$ -adrenergic pathways (Schimmel et al., 1983).  $\beta$ -adrenergic receptor activation acts via the cAMP second messenger cascade to release free fatty acids from lipid stores to be used as substrates in the tricarboxylic acid cycle, and activates an uncoupling protein unique to brown fat mitochondria that can shunt the mitochondrial proton gradient. The physiological uncoupling of oxidation from ATP production by the mitochondrial uncoupling protein leads to rapid oxidation of substrate and large increases in heat production. The resultant increase in metabolic rate is used to regulate body temperature and body weight. Increases in metabolism induced by  $\alpha$ -adrenergic stimulation involve little if any direct mitochondrial uncoupling (Mohell et al., 1987), but rather seem to result from activation of  $\text{Ca}^{2+}$ - and  $\text{H}^+$ -regulating systems.  $\alpha$ -adrenergic receptor stimulation increases  $^{45}\text{Ca}^{2+}$  efflux from prelabeled brown fat cells (Connolly et al., 1984), and norepinephrine stimulation of  $\alpha_2$  receptors in brown fat results in cytosolic alkalization evidently via activation of the amiloride-sensitive Na/H exchanger (Giovannini et al., 1988; Horwitz et al., 1989).

Brown fat undergoes a triphasic membrane electrical response when norepinephrine is applied exogenously or via sympathetic nerve stimulation. Norepinephrine-induced changes in membrane potential have been recorded both *in vivo* (Horwitz et al., 1969, 1971; Flaim et al., 1977; Horwitz and Hamilton, 1984) and *in vitro* (Girardier et al., 1968; Krishna et al., 1970; Williams and Mathews, 1974*a, b*; Fink and Williams, 1976; Girardier and Schneider-Picard, 1983; Schneider-Picard et al., 1985; Hamilton et al., 1988). Although the duration and amplitude of the membrane electrical response vary depending on the concentration and mode of stimulus application, the general response to a maximal norepinephrine stimulation in brown adipose tissue is characterized by three phases: a transient 25-mV depolarization occurring within seconds of stimulation and lasting 10–30 s, a repolarization and hyperpolarization of 5–10 mV lasting 2–5 min, and a final 20–25 mV depolarization that lasts 20–30 min. The initial depolarization is mainly activated by  $\alpha$ -adrenergic agonists and precedes the increase in metabolism (Girardier and Schneider-Picard, 1983). The hyperpolarization has been observed with both  $\alpha$ - and  $\beta$ -adrenergic agonists and can be partially blocked by both  $\alpha$ - and  $\beta$ -adrenergic antagonists (Girardier and Schneider-Picard, 1983; Horwitz and Hamilton, 1984; Schneider-Picard et al., 1985), but it has been termed a  $\beta$ -adrenergic response because it is larger when activated by  $\beta$  agonists (Horwitz and Hamilton, 1984) and because it occurs simultaneously with the metabolic increase (Girardier and Schneider-Picard, 1983). The second sustained depolarization is largely a  $\beta$ -adrenergic response and lasts as long as the cell is metabolically active (Girardier and Schneider-Picard, 1983; Horwitz and Hamilton, 1984).

The strong temporal correlation between the electrical responses and the metabolic responses of brown fat, and the fact that many manipulations that affect the electrical response also affect the metabolic response (Nedergaard, 1981), suggest a relationship between norepinephrine-induced ion fluxes and thermogenesis. To test this, one must know the ionic basis of the membrane electrical response. Because of the high respiration rates and small cytosolic volume of brown fat cells, studies with

radiolabeled ion fluxes have provided only indirect and sometimes conflicting information about the underlying ionic events (Connolly et al., 1984, 1986; Nanberg et al., 1984, 1985; LaNoue et al., 1986; Giovannini et al., 1988). Microelectrode recordings in brown fat cells have been hampered by electrical coupling of the cells (Sheridan, 1971) and poor perfusional access to the cells. In previous experiments we avoided these problems by recording from isolated cultured brown fat cells using the whole-cell patch voltage-clamp technique (Lucero and Pappone, 1989a). These experiments demonstrated the presence of a delayed-rectifier-type K conductance in brown fat cells. However, we could elicit no membrane responses to norepinephrine under these conditions. In the present experiments, we used the "perforated-patch" technique (Horn and Marty, 1988) to measure membrane responses to norepinephrine. We find that cultured brown fat cells respond to norepinephrine with membrane conductance changes that can be activated by both  $\alpha$ - and  $\beta$ -adrenergic agonists. In addition, we observed oscillating hyperpolarizations of the membrane potential reflecting activation of a novel K channel, unique in that it can be blocked by tetraethylammonium (TEA), 4-aminopyridine (4AP), apamin, and charybdotoxin (CTX). Preliminary accounts of this work have appeared (Lucero and Pappone, 1989b; Horwitz et al., 1989).

## METHODS

### *Cells*

Our methods for brown fat cell isolation and culture have been described previously (Lucero and Pappone, 1989a). Briefly, brown adipocytes from interscapular brown fat pads of cold-stressed neonatal Osborne-Mendel or Sprague-Dawley rats were isolated by collagenase digestion (Fain et al., 1967). Cells were plated on acid-cleaned glass coverslips in 35-mm plastic culture dishes in medium consisting of Dulbecco's modified Eagle medium (DMEM), 5% fetal bovine serum (Gibco Laboratories, Grand Island, NY), 0.2 U/ml insulin, and 10  $\mu$ l/ml antibiotic antimycotic solution consisting of 100  $\mu$ g/ml penicillin, 0.1 mg/ml streptomycin, and 0.25  $\mu$ g/ml amphotericin B. All chemicals were from Sigma Chemical Co., St. Louis, MO unless stated otherwise. Cells were used for electrophysiological experiments after 1–23 d in culture.

### *External Solutions*

The external solutions used during electrophysiological studies of cultured brown fat cells contained a 25 mM sodium-bicarbonate buffer. To maintain a pH of 7.4, the solutions were gassed with 95% O<sub>2</sub>/5% CO<sub>2</sub> and continuously perfused over the cells at a rate of ~0.25 ml/min. The standard external solution was Na-Krebs, consisting of 120 mM NaCl, 4.5 mM KCl, 2 mM CaCl<sub>2</sub>, 0.5 mM MgCl<sub>2</sub>, 25 mM NaHCO<sub>3</sub>, 0.7 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.3 mM NaH<sub>2</sub>PO<sub>4</sub>, 20 mM glucose, and 0.1% fatty acid free bovine serum albumin. The K-Krebs and *N*-methyl-D-glucamine (NMDG)-Krebs were identical to the Na-Krebs except that either 120 mM KCl or 120 mM NMDG-Cl were substituted for 120 mM NaCl. The 0-Ca Krebs consisted of Na-Krebs plus 5 mM TMA<sub>2</sub>EGTA, with zero added Ca<sup>2+</sup>. A high Na-Krebs was made by adding 20 mM NaCl salt to Na-Krebs. All external solutions had a pH of 7.4 and an osmolarity of 290–300 mosmol except the high Na-Krebs and TEA-Krebs, which were 315 mosmol. Experiments were done at room temperature, ~22°C.

### *Internal Solutions*

The internal pipette solution used in these studies contained 130 mM K-aspartate, 20 mM KCl, 5 mM NaCl, 1 mM MgCl<sub>2</sub>, 0.5 mM TMA-HEPES, and 0.1 mM K<sub>2</sub>EGTA. The pH of the internal solution was adjusted to 7.2 before filtering and addition of nystatin.

### *Agonists and Antagonists*

All of the adrenergic agonists (norepinephrine, isoproterenol, phenylephrine) or the adrenergic antagonist (propranolol) used in these experiments were made up fresh daily in a Na-Krebs solution at a concentration of 0.25 mg/ml. The stock solutions were kept at 5°C until further dilution in the experimental bath solution by addition of 100  $\mu$ l of the final agonist solution directly to the 400  $\mu$ l chamber. Although the exact concentration of agonist reaching the cell is unknown, we estimate a minimum fourfold dilution since the bath was continually being perfused with agonist-free solution. The durations of the exposures to agonist are not known with precision, but the bath solution was cleared of added agonist in <5 min. A control addition of 100  $\mu$ l of the bath solution without agonist before each experiment showed no effect of the addition procedure on electrical recordings.

### *Blockers*

TEA-Krebs and 4AP-Krebs were made by adding 20 mM TEA-chloride salt (Kodak Chemical Co., Rochester, NY) or 5 mM crystalline 4-AP to Na-Krebs. A stock solution of 20  $\mu$ M apamin in Na-Krebs was kept frozen until further dilution and use. Purified CTX (a gift from E. Moczydlowski, Department of Pharmacology, Yale University) was kept frozen in a 20  $\mu$ M stock in Na-Ringers solution until dilution with Na-Krebs just before use.

### *Perforated-Patch Recording*

Our electrophysiological measurements used the perforated-patch technique (Horn and Marty, 1988) on cultured brown fat cells. This technique uses the antibiotic nystatin to form small pores in the membrane under the electrode. These pores allow small monovalent ions to pass but they prevent movement of larger molecules (Russell et al., 1977). Thus one has electrical access to the entire cell with minimal dialysis of the cytoplasm, avoiding the loss of normal intracellular constituents that occurs in the traditional whole-cell mode of patch-clamp recording (Hamill et al., 1981).

A 500 mg/ml stock solution of nystatin dissolved in dimethylsulfoxide (DMSO) was added to the internal solution to give a final nystatin concentration of 50  $\mu$ g/ml. Nystatin is much more soluble in DMSO than in the methanol used by Horn and Marty (1988). To insure homogenous distribution of the nystatin-DMSO, the pipette solution also contained a dispersing agent, 0.05% Pluronic F-127 (Molecular Probes, Inc., Eugene, OR). The nystatin-DMSO stock solution was made up fresh daily; the Pluronic F-127 was made in a stock solution of 25 mg/ml Pluronic in DMSO, heated at 37°C until it dissolved (~10 min) and added to the pipette solution just before adding the nystatin. Instead of perfusing the pipette solution with nystatin we back-filled the pipette with the nystatin internal solution and then vacuum-filled just the tip of the pipette with nystatin-free internal solution. This allowed us to make G $\Omega$  seals, which were difficult to obtain when nystatin was present at the tip, and gave more rapid access of nystatin to the membrane than was possible with our pipette perfusion system.

Patch electrodes were pulled from Dow-Corning glass (7052; Garner Glass, Claremont, CA), coated with Slygard, and fire polished to have resistance of 2–4 M $\Omega$  when filled with pipette solution. With the pipette immersed in the bath solution the current signal was bal-

anced to zero, but the +9 to +14 mV junction potential between the pipette and bath solutions was not corrected. After a G $\Omega$  seal formed, the pipette capacitance of ~5 pF was nulled electronically. The measured cell capacitance increased slowly as the nystatin diffused to the electrode tip and inserted into the patch membrane. Typically, within 15–20 min after G $\Omega$  seal formation, the series resistance had decreased to <50 M $\Omega$  and on some cells (7/59) the access resistance was <20 M $\Omega$ . Because of the high access resistance, the capacity current transient during steps in voltage had a slow time course and often could not be removed from the current record with our compensation circuitry.

On cells in which the capacitance transient was too slow to compensate there is a vertical offset of the current trace in the positive direction during applied ramps of voltage (e.g., see Figs. 2 B, 5 C and D, 6 A). We did not correct for this offset, so the values for reversal potential of the leak current are not known. Reversal potentials of the norepinephrine-induced currents in Na-Krebs were determined from the intersection of the current records during ramps with and without norepinephrine. In K-Krebs, we extrapolated a linear regression line fitted to the inward currents between –60 and –40 mV. At these potentials, the current was quite large (200–400 pA), and with an average series resistance of 30 M $\Omega$ , caused a –6 to –12 mV shift in the extrapolated reversal potential (e.g., see Fig. 6 B). Resting membrane potentials were measured in current clamp using the perforated-patch technique. The average resting membrane potential of cultured brown fat cells was  $-48 \pm 1$  mV ( $n = 56$ ).

#### *Data Acquisition and Analysis*

Perforated-patch voltage-clamp and current-clamp experiments were run on-line using a Cheshire Data Interface and LSI 11/73 computer system (Indec Systems, Inc., Sunnyvale, CA) to deliver command voltages, and to sample and store data during applied steps or ramps of voltage. The membrane current signal was sampled by a 12-bit A/D converter and filtered at 380 Hz using an 8-pole Bessel filter (Frequency Devices, Inc., Haverhill, MA). Membrane current was filtered at 3 kHz and recorded continuously throughout the experiments using a modified digital audio processor and video cassette recorder (Bezanilla, 1985; Unitrade, Philadelphia, PA). These records were played back on a chart recorder for analysis. All averages are expressed  $\pm$  the standard error.

## RESULTS

### *Cultured Brown Adipocytes Respond Electrically to Norepinephrine*

Our electrophysiological studies using the perforated-patch technique show that day 1–23 cultured brown fat cells are capable of responding to physiological concentrations of norepinephrine with changes in membrane permeability to K<sup>+</sup> and Na<sup>+</sup>. In addition, indirect evidence in the form of norepinephrine-induced oscillatory K<sup>+</sup> currents suggests that internal Ca<sup>2+</sup> levels oscillate when norepinephrine stimulates cultured brown fat cells. Characterization of the oscillatory norepinephrine-activated K channels will be the major emphasis of this paper, but we will begin by briefly describing all of the observed membrane responses to norepinephrine.

### *Norepinephrine Responses*

Membrane current responses to norepinephrine in cultured brown adipocytes are dependent on intact second messenger systems. In experiments using the whole-cell voltage-clamp technique on cultured brown fat cells, (Lucero and Pappone, 1989a),

we were unable to elicit any current response to norepinephrine, even when we added second messengers back to the internal solution and varied the buffering capacity for internal  $\text{Ca}^{2+}$  and pH. Presumably, factors essential for a norepinephrine response are dialyzed out during whole-cell recording.

In the present study, cultured brown fat cells voltage clamped using the perforated-patch technique (Horn and Marty, 1988), which leaves the cytosol intact, exhibited a variety of current responses when stimulated with norepinephrine. Current responses occurred within seconds of norepinephrine addition in 80% of the brown fat cells tested. Some cells (6/38), like the one in Fig. 1, responded with both inward and outward currents. A few cells (3/38) had only inward current responses, while the majority of brown fat cells examined (29/38) had only outward current

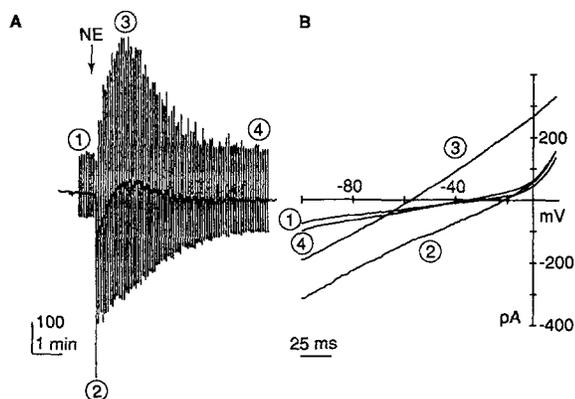


FIGURE 1. Norepinephrine induced both inward and outward currents in a cultured brown fat cell voltage clamped using the perforated-patch technique. (A) The current response to  $<7 \mu\text{M}$  norepinephrine added to a cell voltage clamped at  $-60 \text{ mV}$  is shown on a slow time scale. The spikes are currents elicited by 175-ms ramps of voltage from  $-100$  to  $+20 \text{ mV}$  applied at 2-s intervals. The

numbers designate the ramp current traces shown in B. (B) Superimposed currents during voltage ramps at different times in the norepinephrine response on a faster time scale. These current traces show: 1, control current before norepinephrine addition; 2, the peak of the inward current; 3, the peak of the outward current; and 4, return to control current levels. Slope conductances were measured for each trace by a linear regression fit of the current between  $-90$  and  $-40 \text{ mV}$ . The cell was perfused with Na-Krebs; the pipette contained a K-aspartate + nystatin solution. Each of the following figures (Figs. 2–8) use data from different cells, but the protocols were the same as described here unless stated otherwise.

responses to norepinephrine, like those in Fig. 4 (see below). The duration and amplitude of the current responses were also quite variable. In Fig. 1 an inward current appeared within seconds of adding  $7 \mu\text{M}$  norepinephrine to the perfusing bath with an estimated concentration of  $<1.5 \mu\text{M}$  norepinephrine reaching the cell. The inward current rapidly reached a peak of  $135 \text{ pA}$  then declined in  $30 \text{ s}$ . It was followed by an outward current of  $70 \text{ pA}$ , which returned to control levels in  $4 \text{ min}$ . In other brown fat cells the inward current was sustained for  $4 \text{ s}$  to more than  $12 \text{ min}$  with peak amplitudes ranging from  $20$  to  $350 \text{ pA}$ , while the outward currents lasted  $0.5$ – $7 \text{ min}$  with peak amplitudes of  $30$ – $220 \text{ pA}$ .

The conductance changes during norepinephrine stimulation varied considerably from cell to cell. The spikes in the current record of Fig. 1 A are currents elicited by

ramps of voltage from  $-100$  to  $+20$  mV. Comparison of the ramp currents before and after norepinephrine addition shows that both the inward and outward currents result from increases in membrane conductance. Fig. 1 *B* shows these ramp currents at a faster time scale. The labels in Fig. 1 *A* show at what point each corresponding ramp record in Fig. 1 *B* was taken. The slope of ramp current 1, taken before norepinephrine application, gave a resting cell membrane conductance of 8 nS. After norepinephrine addition, the cell's conductance increased almost 4.5-fold to 35 nS in 2, taken during the peak of the inward current. The norepinephrine-induced currents had a reversal potential near 0 mV, as shown by the intersection of ramp currents 1 and 2. In 3 the outward conductance increased to a peak value of 20 nS, and this conductance increase was accompanied by a negative shift in reversal potential. These effects of norepinephrine were transient, and the conductance returned to control levels in 4. The inward currents in other brown fat cells were associated with

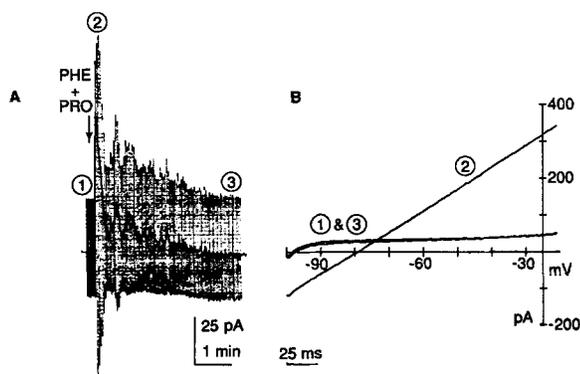


FIGURE 2.  $\alpha$ -Adrenergic agonists activate an oscillating outward  $\text{K}^+$  current. (A) Outward currents activated in response to addition of  $<10 \mu\text{M}$  phenylephrine +  $<1 \mu\text{M}$  propranolol to the Na-Krebs bath. 1, 2, and 3 indicate the ramp current traces shown in B. (B) Superimposed currents recorded during voltage ramps from  $-100$  to  $-20$  mV. The reversal potential of the phenylephrine-induced current was taken as the intersection of currents 1 and 2 ( $-74$  mV). Membrane currents returned to control levels in 3.

two to eightfold increases in conductance, while during outward current generation conductance increased 2–40-fold. Although the norepinephrine-induced currents varied greatly in amplitude, reversal potential, and time course from cell to cell, these features remained relatively constant with repeated agonist applications in the same cell (e.g., see Figs. 7 and 8). The major changes seen with repeated applications of norepinephrine to the same cell were gradual decreases in the amplitude of the current responses and in the number of current oscillations (see below).

#### $\alpha$ - and $\beta$ -Adrenergic Responses

Norepinephrine activates both  $\alpha$ - and  $\beta$ -adrenergic receptors on brown fat cell membranes (Svoboda et al., 1979; Mohell et al., 1983). We tested whether the inward and outward norepinephrine-induced currents were due to activation of different receptor types by examining the membrane responses to specific adrenergic agonists. In Fig. 2 *A*, an outward current was elicited within seconds of adding of 10

$\mu\text{M}$  phenylephrine (an  $\alpha$ -adrenergic agonist) and  $1 \mu\text{M}$  propranolol (a  $\beta$ -adrenergic antagonist) to the perfusing bath. The outward current reached a peak amplitude of  $90 \text{ pA}$  and oscillated several times during the 6-min response. As in the norepinephrine response, the outward current was due to a substantial (21-fold) conductance increase shown in the ramp records of Fig. 2B. As for the norepinephrine responses, the membrane currents induced by  $\alpha$ -adrenergic stimulation were variable. Some cells (4/11) had only inward currents, two had both inward and outward currents, while the rest had oscillating outward currents (5/11). The  $\alpha$ -adrenergically induced current in Fig. 2 reversed at  $-75 \text{ mV}$  and the average uncorrected reversal potential for the  $\alpha$ -adrenergic outward current in Na-Krebs was  $-72 \pm 3 \text{ mV}$  ( $n = 7$ ), close to the expected  $\text{K}^+$  reversal potential. Additional evidence that the phenylephrine-induced outward current was carried mainly by  $\text{K}^+$  comes from experiments in which the extrapolated reversal potential of the phenylephrine-

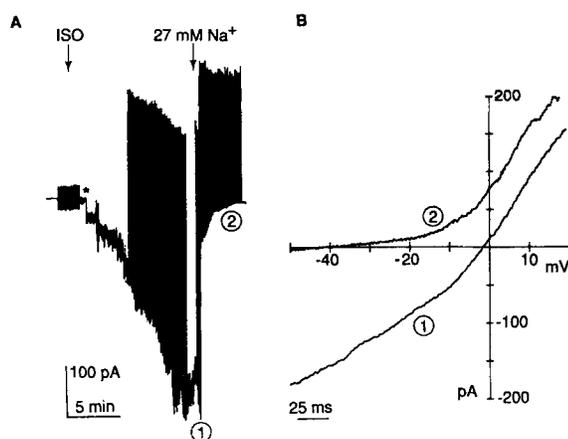


FIGURE 3. Isoproterenol ( $<1 \mu\text{M}$ ) activated a large sustained inward current. (A) Isoproterenol was added to the bath at the first arrow. The \* indicates a 1-min break in the otherwise continuous record of the isoproterenol-activated current. At the second arrow, the bath was changed to NMDG-Krebs. (B) Superimposed currents are shown during the isoproterenol response before (1) and after (2) changing to a low  $\text{Na}^+$  bath solution.  $E_{\text{Na}}$  in Na-Krebs =  $+85 \text{ mV}$ .  $E_{\text{Na}}$  in NMDG-Krebs =  $+43 \text{ mV}$ . The actual shift in reversal potential was  $-38 \text{ mV}$ .

induced current shifted by  $+64 \text{ mV}$  when all the external  $\text{Na}^+$  was replaced with  $\text{K}^+$ .

We examined  $\beta$ -adrenergic responses in cultured brown fat cells by stimulating with isoproterenol.  $\beta$ -adrenergic-induced currents were markedly different from the  $\alpha$ -adrenergic response. We never observed an outward current with isoproterenol, instead the cells either did not respond at all (2/5 cells) or the response was characterized by a slow onset (1–3 min), sustained (20–30 min) inward current with a conductance increase ranging from 2–11-fold (3/5 cells). The  $\beta$ -adrenergic responses were very difficult to characterize because of the slow time course of the response and its resemblance to cell deterioration. The current trace in Fig. 3A shows the isoproterenol-activated current on a slow time scale. The inward current reached a peak amplitude of  $\sim 400 \text{ pA}$  in  $\sim 12 \text{ min}$ . To test whether the isoproterenol-induced current was carried by  $\text{Na}^+$  we changed the bath from Na-Krebs to a

solution where all but 27 mM of the  $\text{Na}^+$  was replaced by the larger cation  $\text{NMDG}^+$ . From the Nernst equation one would predict a  $-42$  mV shift in reversal potential for a  $\text{Na}^+$ -selective conductance. The actual shift of  $-38$  mV is very close to that expected for a  $\text{Na}^+$  conductance as shown in Fig. 3 *B*. In addition, the conductance decreased by 5.6-fold in the low  $\text{Na}^+$  bath solution. These data indicate that the inward current in response to isoproterenol is at least in part carried by  $\text{Na}^+$ . Other ions are probably also involved, since if the  $\beta$ -adrenergic currents were carried by a strictly  $\text{Na}^+$ -selective channel the reversal potential in Na-Krebs would be much more positive than the average uncorrected value of  $-9 \pm 6$  mV ( $n = 3$ ).

Our results using specific agonists indicate that the complex norepinephrine-induced currents may have at least three components: transient  $\alpha$ -adrenergic inward

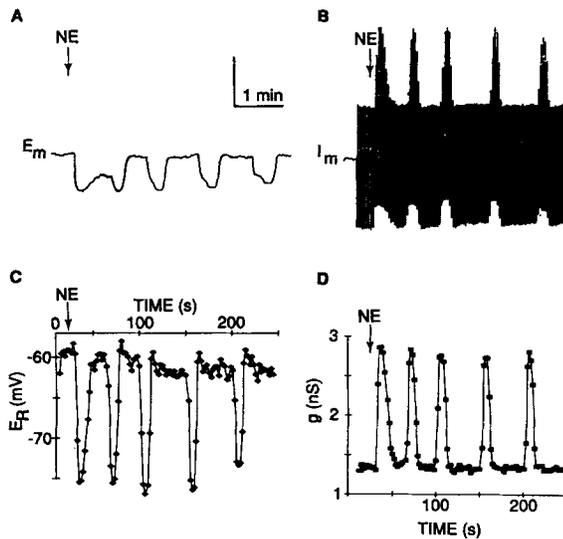


FIGURE 4. Hyperpolarizing responses to norepinephrine follow the same time course as outward K currents, conductance increases, and changes in reversal potential. (A) Current-clamp records of norepinephrine-induced oscillations in membrane voltage from a brown fat cell using the perforated-patch technique. (B) Current records from the same cell in voltage clamp show oscillating outward currents. (C) Changes in reversal potential were plotted vs. time for the currents in *B*. (D) Conductance changes were plotted vs. time for the currents in *B*. The vertical scale bar represents 100 mV and 100 pA for the records in *A* and *B*, respectively. The time scale applies to both *A* and *B*.

and outward currents and  $\beta$ -adrenergic sustained inward currents. The cell-to-cell variability in norepinephrine response is probably due in part to differences in the amount of each current type present in the cell. However, by far the most frequent response to norepinephrine was an oscillating outward current. The remainder of this paper characterizes this current further.

#### *The Norepinephrine-induced Outward Current*

*Activation of the current.* Both norepinephrine and the  $\alpha$ -adrenergic agonist phenylephrine activated oscillating outward currents in cultured brown fat cells. We examined the oscillations in both voltage and current clamp. Fig. 4 *A* shows a con-

tinuous voltage record from a brown fat cell in current clamp with a resting membrane potential of  $-40$  mV. Within seconds of norepinephrine addition, the membrane potential transiently hyperpolarized by  $-40$  mV. Hyperpolarizing oscillations in membrane potential continued for 7 min with a period of  $\sim 1$  oscillation/min. The hyperpolarizing oscillations varied from cell to cell in amplitude ( $-9$  to  $-40$  mV), period (0.4–6 oscillations/min), and duration (0.5–7 min). The oscillating hyperpolarizations in membrane potential could have been due to the turning on and off of voltage-gated channels. However, as shown in Fig. 4 B, oscillations in outward current when norepinephrine was added to the same cell voltage clamped at  $-60$  mV were similar in time course to the voltage oscillations in current clamp, arguing against a voltage-controlled activation process.

The increases in outward current are associated with increases in membrane conductance as shown in the plot of conductance vs. time for the same response in Fig. 4 D. The conductance increase appears to be  $K^+$  selective since the uncorrected

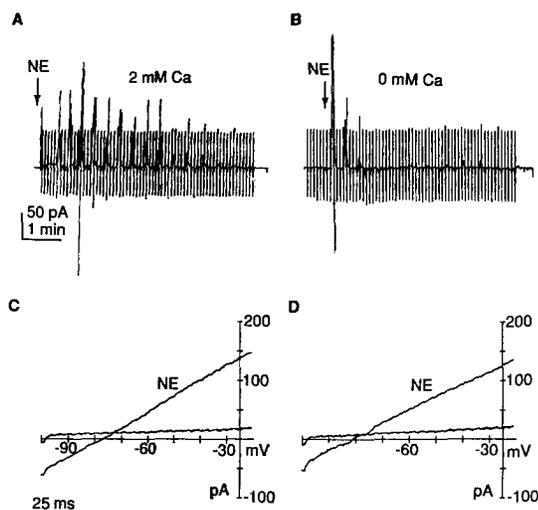


FIGURE 5. Outward current responses to norepinephrine were attenuated in the absence of external  $Ca^{2+}$ . Shown are outward oscillating membrane currents recorded in voltage-clamp after addition of norepinephrine in Na-Krebs containing (A) 2 mM  $Ca^{2+}$  or (B) 0  $Ca^{2+}$  + 5 mM EGTA. Currents elicited by voltage ramps before, during, and after the largest oscillation from each of the above responses were essentially the same in (C) 2 mM  $Ca^{2+}$  as in (D) 0  $Ca^{2+}$  + 5 mM EGTA.

reversal potentials also oscillated from a resting value of  $-57$  to  $-77$  mV at the peak of the norepinephrine-induced outward current as shown in Fig. 4 C.

Since many cells show oscillations in their internal  $Ca^{2+}$  levels in response to membrane receptor activation (Rink and Jacob, 1989), and since most cells contain  $Ca^{2+}$ -activated K channels (Hille, 1984), it seems likely that the oscillating outward currents are conducted by  $Ca^{2+}$ -activated K channels. Preliminary data using the fluorescent  $Ca^{2+}$  indicator fura-2, show an increase in internal  $Ca^{2+}$  levels in response to norepinephrine stimulation in cultured brown adipocytes, and the experiments presented below show the conductance increase is to  $K^+$ . It appears that at least the initial internal  $Ca^{2+}$  increase in response to norepinephrine occurs via  $Ca^{2+}$  release from internal stores. Fig. 5, C and D show that the maximal conductance increase was similar when the bath solution contained 2 or 0 mM  $Ca^{2+}$ . However, in the presence of external  $Ca^{2+}$  there were 15 oscillations in the outward current, while in the absence of  $Ca^{2+}$  the same stimulus resulted in only three current oscillations,

suggesting that external  $\text{Ca}^{2+}$  may be important for maintaining the oscillating  $\text{K}^+$  current response.

**Selectivity.** To test whether the norepinephrine-induced outward currents were carried by  $\text{K}^+$  we changed the bathing solution from Na-Krebs containing 4.5 mM  $\text{K}^+$  to K-Krebs containing 130 mM  $\text{K}^+$ . Fig. 6 A shows norepinephrine-induced currents in low external  $\text{K}^+$ . The current activated by norepinephrine reversed at  $-65$  to  $-70$  mV. The average uncorrected reversal potential of the outward currents in Na-Krebs was  $-73 \pm 1$  mV ( $n = 28$ ). Activation of inward  $\text{K}^+$  currents through the voltage-gated K channels present in brown fat cells can be seen in high external  $\text{K}^+$  at potentials positive to  $-20$  mV in Fig. 6 B. The voltage-gated K currents reversed at  $+5$  mV in this experiment and at  $+10$  mV in two other perforated-patch experiments in high  $\text{K}^+$  solution. Since we know from whole-cell patch experiments that the voltage-gated K channels are highly selective for  $\text{K}^+$  (Lucero and Pappone, 1989a) and that the Nernst potential for  $\text{K}^+$  under these ionic conditions is  $-4$  mV, we assume there is a potential offset in these experiments of  $+9$  to  $+14$  mV. This

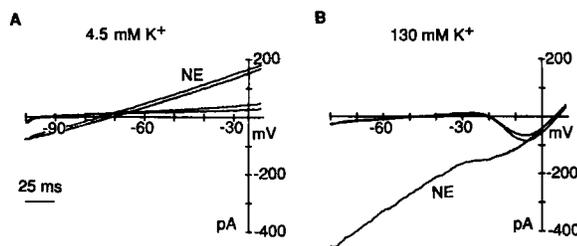


FIGURE 6.  $\text{K}^+$  selectivity of the norepinephrine-induced current. (A) Currents recorded during ramps of voltage between  $-100$  and  $-20$  mV. Those labeled NE were maximal responses seen in response to norepinephrine in Na-Krebs before and after changing the external solution to K-Krebs. (B) Currents elicited by voltage ramps from  $-80$  to  $+10$  mV in K-Krebs before, during, and after the response to norepinephrine.

would suggest that the actual reversal potential for the norepinephrine-induced current in Na-Krebs is  $-87$  mV, close to the  $E_{\text{K}}$  of  $-88$  mV for these solutions. In K-Krebs, the reversal potential for the norepinephrine-induced current extrapolated from the current at negative potentials was  $-3$  mV in the experiment shown in Fig. 6 B. However, the substantial uncompensated series resistance and the large inward currents at negative voltages probably contribute a  $-6$  to  $-12$  mV error in the extrapolated reversal potential measurements, suggesting that the norepinephrine-induced currents actually reverse near  $E_{\text{K}}$ . This view is substantiated by the fact that there is no shift in the reversal potential of the total current in norepinephrine as can be seen in Fig. 6 B. Thus, the reversal potential experiments indicate that channels conducting the norepinephrine-induced outward current are selective for  $\text{K}^+$ . We will therefore refer to them as  $I_{\text{K,NE}}$ .

**K Channel Blockers.** Many different types of K channels can be distinguished based on their susceptibility to block by a variety of compounds (Moczydlowski et

al., 1988; Rudy, 1988; Castle et al., 1989). In the present studies, we found that blockers of both voltage-activated and  $\text{Ca}^{2+}$ -activated K channels blocked the norepinephrine-induced K current. Our previous experiments showed that TEA and 4AP block the voltage-gated K channels in brown fat cells with a  $K_d$  of 1–2 mM (Lucero and Pappone, 1989a). Fig. 7A shows current records of norepinephrine responses from a cell elicited before, during, and after perfusion with solution containing 20 mM TEA. In this and three other experiments, 20 mM TEA completely blocked the outward currents. The block by TEA was readily reversed, and  $I_{\text{K,NE}}$  could again be elicited within 5 min of washing out the TEA. Experiments to control for the increased osmolarity and  $\text{Cl}^-$  concentration in the TEA solution compared

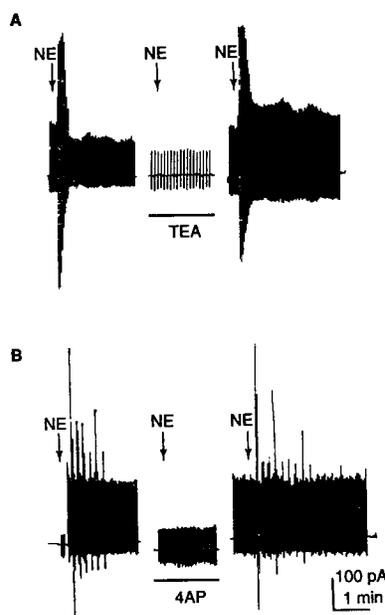


FIGURE 7. Effects of TEA and 4AP on the norepinephrine-induced K currents. (A) Current records showing responses to norepinephrine before, during, and after changing the bath from Na-Krebs to Na-Krebs + 20 mM TEA. Voltage ramps from  $-100$  to  $+20$  mV were applied in the control and wash experiments at 2-s intervals and at 5-s intervals in the presence of TEA (indicated by the solid bar). The current records were not continuous. (B) In a different cell, 5 mM 4AP (indicated by the solid bar) completely blocked  $I_{\text{K,NE}}$  and caused an 18-pA inward shift in the holding current. Ramps of voltage were applied at 2-s intervals throughout.

norepinephrine responses in normal Na-Krebs and in Na-Krebs plus an additional 20 mM NaCl. There were no apparent differences in the current responses in the two solutions. The current records in Fig. 7B show that 5 mM 4AP also completely blocked  $I_{\text{K,NE}}$ . Block by 4AP was also reversible and the norepinephrine-induced currents returned within 5 min of perfusion with 4AP-free solution in three out of four cells.

Blockers of  $\text{Ca}^{2+}$ -activated K channels were also effective in blocking  $I_{\text{K,NE}}$ . The effects of 500 nM apamin are shown in Fig. 8A. In seven experiments apamin completely blocked the outward current response to norepinephrine. Apamin also completely blocked the outward currents elicited by the  $\alpha$ -adrenergic agonist phenylephrine. The recovery from block by apamin was slower and less complete than from TEA or 4AP. In only four out of seven cells did  $I_{\text{K,NE}}$  recover completely, and it took up to 20 min of perfusion with apamin-free solution before  $I_{\text{K,NE}}$  returned to control levels in these cells. CTX also completely blocked the  $I_{\text{K,NE}}$  currents as shown in Fig.

8 B. Because of our small supply of CTX, these experiments were done slightly differently than the other blocker experiments. The perfusion was stopped just before the control norepinephrine addition and restarted when the response ended, after ~2 min. The bath was then changed to 200 nM CTX in Na-Krebs and norepinephrine was again added. There was no outward current in response to norepinephrine stimulation in any of the five CTX experiments. Perfusion with CTX-free solution was restarted when there was no response after 2–3 min. Block by CTX was only slowly reversed, and it took 10–20 min of washing with CTX-free solution before

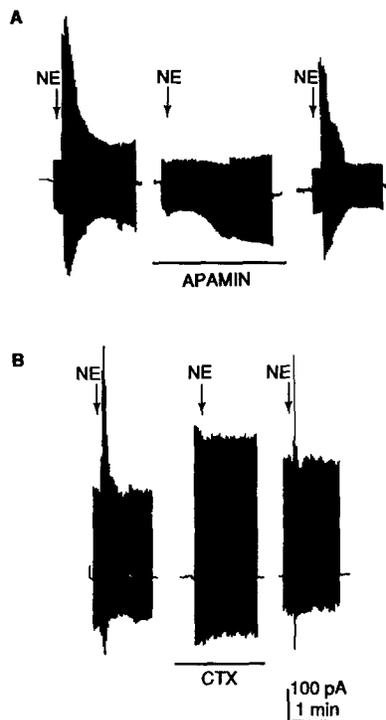


FIGURE 8. Effects of apamin and CTX on norepinephrine-induced K currents. (A) Current records showing that changing the bath from Na-Krebs to Na-Krebs + 500 nM apamin completely blocked the norepinephrine-induced K current. The same protocol was used as in Fig. 7 except that voltage ramps were applied at 2-s intervals throughout. (B) 200 nM CTX also completely blocked the norepinephrine-induced outward current in another cell. In these experiments the perfusion was stopped during application of norepinephrine, and restarted between norepinephrine applications.

$I_{K,NE}$  could again be elicited. However, at least partial recovery from CTX block was observed in three out of five cells.

In two of the six apamin experiments, norepinephrine addition resulted in a slight increase in conductance and the appearance of a small inward current, which was presumably previously masked by the outward currents. Although we have not studied this unmasked inward current enough to determine which ion is involved, its appearance suggests that in the experiments in which we observed only a net outward current, norepinephrine could also be increasing membrane permeability to ions with positive reversal potentials. Fig. 8 B shows that CTX block of  $I_{K,NE}$  also unmasked a small inward current when norepinephrine was applied in this cell.

In all of the 4AP experiments the holding current shifted inward by 12–18 pA in the presence of 5 mM 4AP and returned to control levels after it was washed out. In addition, the resting membrane conductance was lower with 4AP present. Similar

changes in resting membrane properties were observed in two of four cells in 20 mM TEA. Apamin or CTX, however, had no effect on holding current and no consistent effect on resting membrane conductance. There was an increase in the resting conductance in the presence of CTX in the cell of Fig. 8 B, but in the other four cells tested, the resting conductance either decreased slightly or stayed the same as control in the presence of CTX. Variability in resting conductance in the presence of CTX may have been due to pH changes in the bath solution upon stopping perfusion during these experiments. The changes in holding current with 4AP and TEA indicate that they block a portion of the cell's resting conductance, which was probably contributed by voltage-gated K channels that were open at rest. Previously, we found that voltage-gated K channels are blocked by both TEA and 4AP and their voltage dependence indicated that they could contribute to the resting conductance (Lucero and Pappone, 1989a). The lack of effect of CTX and apamin on the holding current indicates that the norepinephrine-activated K channels has a low probability of being open in the absence of agonist under our experimental conditions.

The pharmacology described above indicates that the  $\text{Ca}^{2+}$ -activated K channel is different from the voltage-gated K channel in brown fat because  $I_{\text{K,NE}}$  can be blocked by concentrations of apamin and CTX that had no effect on the voltage-gated channel (Lucero and Pappone, 1989a).  $I_{\text{K,NE}}$  seems to be carried by a novel  $\text{Ca}^{2+}$ -activated K channel since it is blocked by both apamin and CTX, two toxins which have not previously been seen to block the same population of channels (Latorre et al., 1989).

#### *Norepinephrine Effects on Voltage-gated K Channels*

In our previous whole-cell voltage-clamp experiments in cultured brown fat cells we saw no effects of norepinephrine on the voltage-gated K channels. We did not know if this lack of response was a normal property of the channels, or was due to dialysis of some essential cytoplasmic component from the cells, or came about through loss of hormone sensitivity in culture. In the perforated-patch experiments, even in cells with other membrane responses to norepinephrine, norepinephrine still did not directly affect the voltage-gated K channels. As can be seen in Fig. 6 B, there were no obvious changes in voltage dependence of channel activation or kinetics of the voltage-gated K currents in high  $\text{K}^+$  solutions in the presence of norepinephrine. In some instances we did observe an increase in amplitude of voltage-gated K currents after a norepinephrine response but we were unable to determine whether the current increase was due to norepinephrine or to changes in the clamp properties with time.

#### DISCUSSION

Membrane electrical responses are commonly associated with excitable tissues such as nerve and muscle where they primarily function to rapidly send information over long distances or activate release of neurotransmitters or hormones. Recent studies in nonexcitable tissues suggest that membrane electrical responses are important in a variety of cellular processes such as stimulus-secretion coupling (Petersen and Maruyama, 1984), mitogenesis (Cahalan et al., 1985; Chiu and Wilson, 1989; Koni-

shi, 1989), and volume regulation (Christensen, 1987). The application of the patch-clamp technique (Hamill et al., 1981) to nonexcitable cells is largely responsible for the rapid increase in our understanding of the role of membrane currents in these cells.

This paper presents the first recordings of membrane electrical responses to norepinephrine in single cultured brown fat cells using a modified patch-clamp technique, the "perforated patch" (Horn and Marty, 1988). In our previous experiments we saw no membrane responses to norepinephrine in cultured brown fat cells using the traditional whole-cell configuration of the patch clamp (Lucero and Pappone, 1989a). Using the virtually noninvasive perforated patch we consistently find vigorous membrane responses to norepinephrine in our cultured cells. Since the difference between this method and traditional whole-cell recording is that larger molecules are prevented from diffusing out of the cell into the pipette, we assume that in our earlier experiments an essential factor or factors for the membrane norepinephrine response were dialyzed out upon rupturing the membrane under the patch pipette. Thus it seems that cytoplasmic components are essential in eliciting the membrane electrical response to norepinephrine, and that second messenger systems may be involved. In our previous experiments there were no effects of internally applied cyclic nucleotides on the membrane properties of brown fat cells. The most likely possibility is that norepinephrine stimulates inositol metabolite-mediated activation of membrane conductances, at least in part through release of  $\text{Ca}^{2+}$  from intracellular stores and possibly through changes in intracellular pH as well (Horwitz et al., 1989).  $\text{IP}_3$  levels are known to increase in brown fat in response to adrenergic activation (Garcia-Sainz et al., 1980; Schimmel et al., 1983, 1986; Nanberg and Putney, 1986), and  $\text{IP}_3$ -induced increases in internal  $\text{Ca}^{2+}$  have been observed in many cells (Woods et al., 1986; Berridge, 1987; Berridge and Galione, 1988).

#### *Membrane Potential Responses*

The membrane electrical response of brown fat tissue to norepinephrine is composed of three phases, beginning with a transient depolarization of  $\sim 25$  mV, which occurs within seconds of norepinephrine addition and lasts for 10–30 s. This first phase is largely mediated by  $\alpha$ -adrenergic agonists and precedes the thermogenic increase in metabolism. The second phase is a repolarization and hyperpolarization of the membrane by 5–10 mV. This phase lasts from 30–120 s and occurs at the same time as the increase in metabolism (Girardier and Schneider-Picard, 1983). The third phase of the electrical response is a slow sustained depolarization of  $\sim 25$  mV that lasts as long as the cell is metabolically active (Horwitz and Hamilton, 1984). Application of norepinephrine to cultured brown fat cells in current clamp induced both depolarizations and hyperpolarizations of the membrane potential that were similar in amplitude and duration to membrane potential changes observed in microelectrode studies on brown adipose tissue *in vivo* or *in vitro* (Horwitz and Hamilton, 1984; Hamilton et al., 1988). All three components of the complete triphasic response seen in brown adipose tissue were only rarely observed in individual cultured cells, however, each component of the tissue response was present in a subset of the cells. The potential responses varied in form, amplitude, and

duration from cell to cell, but were similar within an individual cell with repeated agonist applications. In brown adipose tissue the cells are electrically coupled via gap junctions (Sheridan, 1971), so the membrane response recorded with a microelectrode in one cell reflects the averaged electrical response of all the cells to which it is coupled. The tissue response then may represent a consistent population average composed of more variable individual cell responses.

#### *Membrane Current Responses*

Voltage-clamp experiments showed that both the depolarizing and hyperpolarizing voltage responses were due to increases in membrane conductance. As for the membrane potential responses, there was a great deal of variation in current response properties, with each cell showing a characteristic "signature" response to norepinephrine (Prentki et al., 1988).

*Inward currents.* Large (300–400 pA), sustained (20–30 min), inward currents were activated by the  $\beta$ -adrenergic agonist isoproterenol. These currents were carried largely by  $\text{Na}^+$  and may have been conducted by the nonselective cation channel previously observed in inside-out patches from cultured brown fat cells (Siemen and Reuhl, 1987). Smaller, transient inward currents were occasionally seen in response to the  $\alpha$ -agonist phenylephrine or in the presence of K channel blockers. Because of the transient nature and rarity of these currents, we have not determined their ion selectivity. There is evidence against  $\text{Ca}^{2+}$  carrying this current since  $\text{Ca}^{2+}$  channel blockers or  $\text{Ba}^{2+}$  substitution for external  $\text{Ca}^{2+}$  do not effect the  $\alpha$ -mediated depolarization (Hamilton et al., 1988).

*Outward currents.* The most frequent current response to norepinephrine in cultured cells was an oscillating outward current carried by  $\text{K}^+$  ions which we have termed  $I_{\text{K,NE}}$ . Hyperpolarizing oscillations in membrane potentials have been previously reported in microelectrode studies of brown fat (Williams and Matthews, 1974a, b; Horwitz and Hamilton, 1984), although their veracity was questioned. We found that the oscillating hyperpolarizations were due to a selective increase in the membrane  $\text{K}^+$  conductance most probably in response to changes in internal  $\text{Ca}^{2+}$  concentration. Stimulation increases  $^{45}\text{Ca}^{2+}$  efflux from preloaded cells (Connolly et al., 1984), which suggests that cytoplasmic  $\text{Ca}^{2+}$  levels rise during the thermogenic response. In addition, our preliminary fura-2 experiments also show a transient rise in internal  $\text{Ca}^{2+}$  when cultured brown fat cells are stimulated with norepinephrine (Pappone, P. A., and M. T. Lucero, unpublished observations). Since we found that external  $\text{Ca}^{2+}$  was not necessary to elicit the initial components of the current response at least some of the increased cytoplasmic  $\text{Ca}^{2+}$  must come from intracellular stores, either in the mitochondria or endoplasmic reticulum. Adrenergic stimulation of brown fat reduces  $\text{Ca}^{2+}$  uptake by mitochondria, presumably because of the decreased electromotive force across the inner mitochondrial membrane resulting from the uncoupling process (Connolly and Nedergaard, 1988).

In other cell types a direct correlation between oscillating outward  $\text{K}^+$  currents like those we see in cultured brown fat and oscillations in internal  $\text{Ca}^{2+}$  has been demonstrated (Igusa and Miyazaki, 1986; Ueda et al., 1986) and the  $\text{Ca}^{2+}$ -activated K channel kinetics can be used as a sensitive indicator of the submembranous  $\text{Ca}^{2+}$  concentration (Rink and Jacob, 1989). Thus it seems likely that internal  $\text{Ca}^{2+}$  levels,

at least near the membrane, fluctuate in response to norepinephrine. It has been shown that activation of  $\alpha_1$ -adrenergic receptors in brown fat increases the generation of  $IP_3$  (Garcia-Sainz et al., 1980; Schimmel et al., 1983, 1986; Nanberg and Putney, 1986). Fluctuations in internal  $Ca^{2+}$  could result from  $\alpha$ -adrenergic activation of the  $IP_3$  pathway causing an oscillating release of  $Ca^{2+}$  from internal stores as has been shown in other cell types (Woods et al., 1986; Berridge, 1987; Berridge and Galione, 1988). We found that  $I_{K,NE}$  still activated in the  $Ca^{2+}$ -free solutions but the oscillations were attenuated suggesting that a refilling of internal stores by external  $Ca^{2+}$  may be necessary for a sustained response (Jacob et al., 1988).

$I_{K,NE}$  was activated by  $\alpha$ -adrenergic stimulation and was not observed when the  $\beta$ -adrenergic agonist isoproterenol was applied, although we measured only a few  $\beta$ -adrenergic responses. This pharmacology agrees with previous measurements in brown fat. Schneider-Picard et al. (1985) using extracellular ion-sensitive microelectrodes measured two outward fluxes of  $K^+$ , a fast transient efflux that was blocked by  $\alpha$ -adrenergic antagonists and a second slow sustained  $K^+$  efflux that was blocked by preincubation with  $\beta$ -adrenergic antagonists. In addition, Nanberg et al. (1984, 1985) found an apamin-sensitive  $^{86}Rb^+$  efflux that can be activated by specific  $\alpha$ -adrenergic stimulation of isolated brown adipocytes. It seems likely from our results that most of the transient  $K^+$  efflux in these experiments was due to  $I_{K,NE}$  while the sustained  $K^+$  efflux may have additional contributions from currents through voltage-gated K channels (Lucero and Pappone, 1989a) during the sustained depolarization produced in the  $\beta$ -adrenergic response. In contrast, Horwitz and Hamilton (1984) concluded that the transient hyperpolarization was primarily a  $\beta$ -adrenergic response, suggesting that there may be overlap of the K channel responses. Thus the  $I_{K,NE}$  currents, possibly in combination with the voltage-gated K currents also present, are probably responsible for the hyperpolarizing phase of the membrane response to norepinephrine and might also be active during the sustained depolarizing phase of the response.

We tested the sensitivity of the norepinephrine-induced K current to block by the K channel blockers apamin, CTX, TEA, and 4AP and found surprisingly that all four of the K channel blockers reversibly blocked  $I_{K,NE}$ . This pharmacology is to our knowledge unique, since apamin has previously been reported to block only  $Ca^{2+}$ -activated K channels, and 4AP usually acts on voltage-activated K channels (Rudy, 1988; Castle et al., 1989). Furthermore, in previous experiments apamin and CTX acted on different conductance classes of  $Ca^{2+}$ -activated K channels (Latorre et al., 1989). We have previously shown that the voltage-gated K channels in cultured brown fat cells are blocked by both TEA and 4AP with a  $K_d$  of 1–2 mM, but that neither apamin nor CTX block these K channels (Lucero and Pappone, 1989a). These data, and the lack of any apparent effect of norepinephrine on the voltage-gated K currents, indicate that the voltage-gated and norepinephrine-activated K channels are two distinct channel species.

#### *Metabolic Role of K Channels in Brown Fat*

The work presented in this paper indicates that both  $Ca^{2+}$ -activated and voltage-gated K channels contribute to the membrane potential responses of norepineph-

rine-stimulated brown adipose tissue. Since previous studies (Girardier and Schneider-Picard, 1983; Schneider-Picard et al., 1985) have shown that the hyperpolarizing phase of the triphasic response to norepinephrine occurs during the increase in metabolism, the two events may be related. In preliminary experiments (Pappone, P. A., and M. T. Lucero, unpublished observations) we have measured increases in norepinephrine-induced heat production in acutely isolated brown fat cells and found that with maximal norepinephrine stimulation block of both norepinephrine-activated and voltage-gated K channels decreases the sustained thermogenic response. Thus, functioning K channels may be necessary for a full metabolic response to norepinephrine.

It is likely that K channels affect thermogenesis through effects on volume and pH regulating systems since thermogenesis produces substantial osmotic and hydrogen ion loads on the cells. Activation of thermogenesis involves the generation of high concentrations of free fatty acids through activation of a K<sup>+</sup>-sensitive lipase. The free fatty acids released into the cytoplasm and the products of their oxidation result in a substantial osmotic load on the brown fat cell. This load could be offset in part by K<sup>+</sup> efflux through  $I_{K,NE}$  and voltage-gated K channels. Chloride ion movements may also be involved since brown fat cells have a fairly large resting Cl<sup>-</sup> conductance and Cl<sup>-</sup> is thought to be distributed passively across the membrane (Williams and Matthews, 1974a).

Hydrogen ion regulation is also likely to be important to thermogenesis since the mitochondrial uncoupling protein is very sensitive to pH and the H<sup>+</sup> concentration must be kept low in order for the uncoupling to proceed (Nicholls, 1974). Although stimulated brown fat produces substantial amounts of hydrogen ions, recent studies have shown that brown fat cells are extremely efficient at dumping their proton load and that even during a thermogenic episode the intracellular pH becomes slightly alkaline (Horwitz et al., 1989). This transport of H<sup>+</sup> out of the cell is thought to occur via an amiloride-sensitive Na/H exchanger in the plasma membrane. The Na/H exchanger can be modulated by activation of  $\alpha_2$ -adrenergic receptors (Giovannini et al., 1988) and is dependent on the movement of Na<sup>+</sup> down its electrochemical gradient. Since as much as half of the driving force for Na/H exchange can come from the membrane potential, opening K channels during the thermogenic response may be important in maintaining an electrical driving force for this process.

K channel activity may also be important in long-term regulation of brown fat. During cold acclimation, brown fat becomes both hyperplastic and hypertrophic (Skala and Hahn, 1984; Cunningham and Nicholls, 1987). Currents through K channels in other preparations seem to be essential for activation of mitogenesis (Decoursey et al., 1984; Chiu and Wilson, 1989; Konishi, 1989). Thus K channels in brown fat precursor cells may also play a role in the hyperplasia that occurs during chronic adrenergic stimulation. These crucial roles played by K channels in brown fat cells are likely to be important in other metabolically active cells as well.

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