Modulation of calcium-activated non-selective cation channel activity by nitric oxide in rat brown adipose tissue

Ari Koivisto and Jan Nedergaard

The Wenner-Gren Institute, The Arrhenius Laboratories F3, Stockholm University, S-10691 Stockholm, Sweden

1. Single-channel calcium-activated non-selective cation currents from isolated rat brown fat cells were measured using the inside-out patch configuration of the patch-clamp technique. The existence of a possible modulatory effect of nitric oxide on the putative redox-modulatory site located on the intracellular side of the non-selective cation channel was investigated.

2. The nitric oxide-releasing substances nitroglycerin, sodium nitroprusside, *S*-nitrosocysteine and *S*-nitroso-*N*-acetyl-D,L-penicillamine (all at 100 μM) were able to block channel activity almost completely.

3. In each case the blockade was persistent and could not be washed away. Dithiothreitol (DTT, 2 mM) was able to reverse the blockade to a large extent, whereas oxidized DTT (2 mM) was without effect.

4. It was concluded that nitric oxide can modulate non-selective cation channel activity by oxidizing sulfhydryl groups and that this effect can be reversed by reduction.

A calcium-activated cation non-selective (NSC) channel with a conductance of about 30 pS and rather slow kinetics was originally characterized with the patch-clamp technique in heart muscle cells (Colquhoun, Neher, Reuter & Stevens, 1981). Since then, non-selective cation channels have been described in a wide variety of species and cells (Partridge & Swandulla, 1988; Siemen & Hescheler, 1993), including brown fat cells (Siemen & Reuhl, 1987). The function of this channel in brown adipose tissue is not well known but it may explain (Siemen & Reuhl, 1987) the long-lasting depolarization observed during adrenergic stimulation of the tissue (Girardier, Seydoux & Clausen, 1968; Lucero & Pappone, 1990).

Recently, the presence of a critical sulfhydryl group on the cytosolic side of the non-selective cation channel was suggested. It would seem that this sulfhydryl group is sensitive to oxidation by sulfhydryl-group specific reagents, and that the redox state of the group affects channel gating (Koivisto, Siemen & Nedergaard, 1993). This site is thus analogous to the redox-modulatory site which has been identified in the NMDA-receptor channel complex (Aizenman, Lipton & Loring, 1989). NMDA-receptor channel activity was recently shown to be reversibly modulated by nitric oxide (NO), acting on the sulfhydryl groups of the redox-modulatory site (Lei *et al.* 1992; Lipton *et al.* 1993). In that process, nitric oxide is thought to act as an oxidizer, which leads to disulphide bond formation by interaction of two closely located (vicinal) *S*-nitrosylated thiols. Based on our previous observations of regulatory sulfhydryl groups of the non-selective cation channel (Koivisto *et al.* 1993), we found it possible that non-selective cation channels could be modulated by nitric oxide in a manner similar to that observed for the NMDA-receptor channel.

In order to investigate this possibility, we have examined the ability of nitric oxide to regulate the non-selective cation channel activity in brown adipose tissue. We found that nitric oxide was able to block the activity of the non-selective cation channel, possibly by oxidation of sulfhydryl groups, and that the effect could be reversed by reduction.

METHODS

Cell isolation and maintenance

Young male (80–140 g) Sprague–Dawley rats were killed by inhalation of 100% CO_2, and mature brown adipocytes were isolated from the interscapular depot of this tissue by collagenase (5 mg ml^{-1}) digestion and dispersed into single cells by shaking in extracellular solution (see below) at 37 °C for 35 min. Floating cells were then separated from the collagenase suspension by centrifugation (150 *g*, 10 min) and kept 1–7 days in an incubator at 37 °C and in an 8% CO_2–92% air atmosphere in Dulbecco's modified Eagle's medium (ICN Biomedicals, Inc., Costa Mesa, CA, USA), supplemented with newborn calf serum (10% v/v), insulin (4 nM), sodium ascorbate (25 μg ml^{-1}), glutamine (4 mM), penicillin (50 IU ml^{-1}) and streptomycin (50 μg ml^{-1}). The floating fat cells adhered to the hydrophilic side of Heraeus-Biofoil-25® (Heraeus GmbH, Hanau, Germany), which was placed on the surface of the medium. For the experiments, the biofoil with the attached cells was placed on a Petri dish filled with extracellular solution.

Electrophysiology

We used the inside-out mode of the patch-clamp technique (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). Pipettes were pulled from borosilicate glass (Clark GC-150, Pangbourne, Berkshire, UK) and had resistances between 8 and 12 MΩ. In the displayed recordings, upward deflections denote currents corresponding to cellular outward currents. The currents were recorded with an L/M EPC 7 patch-clamp amplifier (List Medical, Darmstadt, Germany). After the signal had been digitized with a modified pulse code modulator (Sony 601 ESD, Cologne, Germany), the data were stored on video cassettes. The data were transferred by a 12-bit interface (Labmaster TL-1 DMA, Axon Instruments, Foster City, CA, USA) for off-line analysis with a 486-processor-based computer.

In all experiments, both the extracellular and the pipette solution comprised (mM): 134 NaCl, 6 KCl, 1·2 $MgCl_2$, 1·2 $CaCl_2$, and 10 Hepes (pH 7·4, adjusted with NaOH). After excision, the patches were perfused with a solution containing (mM): 130 KCl, 10 NaCl, 1·2 $MgCl_2$, 1·2 $CaCl_2$, and 10 Hepes (pH 7·2, adjusted with KOH). All test substances were added to this solution. Nitroglycerin (Tika Läkemedel, Lund, Sweden) was dissolved in ethanol. Dithiothreitol (DTT), *N*-acetyl-D,L-penicillamine (NAP), *N*-ethylmaleimide (NEM), oxidized dithiothreitol (oxDTT), sodium nitroprusside (SNP; all from Sigma) and *S*-nitroso-*N*-acetyl-D,L-penicillamine (SNAP; Alexis Corporation, Switzerland) were dissolved directly in the perfusion solution. The perfusion system consisted of a 4-channel local application holder (Pharma-Robot, Kiev, Ukraine), which was fed by gravity, each channel being controlled separately by an electromagnetic valve (The LEE Company, Westbrook, CT, USA). All experiments were performed at room temperature (22 °C).

Preparation of *S*-nitrosocysteine

Equimolar amounts of L-cysteine (Sigma) and sodium nitrite $NaNO_2$ (Aldrich) were mixed in distilled water, which had been bubbled with helium (AGA, Stockholm, Sweden) for at least 1 h before use. To this solution, 37% HCl was added to achieve a concentration of 0·5 M with a final *S*-nitrosocysteine concentration of 100 mM. This solution was diluted for experiments, so that the final concentration of the *S*-nitrosocysteine formed in the perfusion solution was 100 μM. Before use, the pH was adjusted to 7·2 with KOH.

Data analysis

The current signal was sampled at a frequency of 5 kHz and was low-pass filtered with a −3 dB frequency of 0·5 kHz by an 8-pole filter with Bessel characteristics (Frequency Devices, Haverhill, MA, USA). Results were analysed by using the pCLAMP 6.01 program (Axon Instruments). Open time probabilities (P_o) were calculated from amplitude histograms with data collected over at least 30 s, according to the following algorithm:

$$P_o = \Sigma(ns_n)/SN,$$

where n is the number of simultaneously active channels, (0 is no channels open, 1 is 1 channel open, etc.), s_n is the number of samples the channel has in the state n, S is the total number of samples in the recording and N is the maximum number of channels detectable during the experiment. Single channel amplitudes were calculated from amplitude histograms fitted by the simplex least-squares algorithm. Results are given as means ± S.E.M. Statistical significance was tested with Student's paired t test.

RESULTS

Characteristics of calcium-activated non-selective cation channels in brown fat cells

Non-selective cation channels are the predominant channel type observed in inside-out patches from brown fat cells (Siemen & Reuhl, 1987). They are characterized by being activated in Ca^{2+}-containing solutions and inactivated by Ca^{2+} chelation. They have slow kinetics, a conductance of

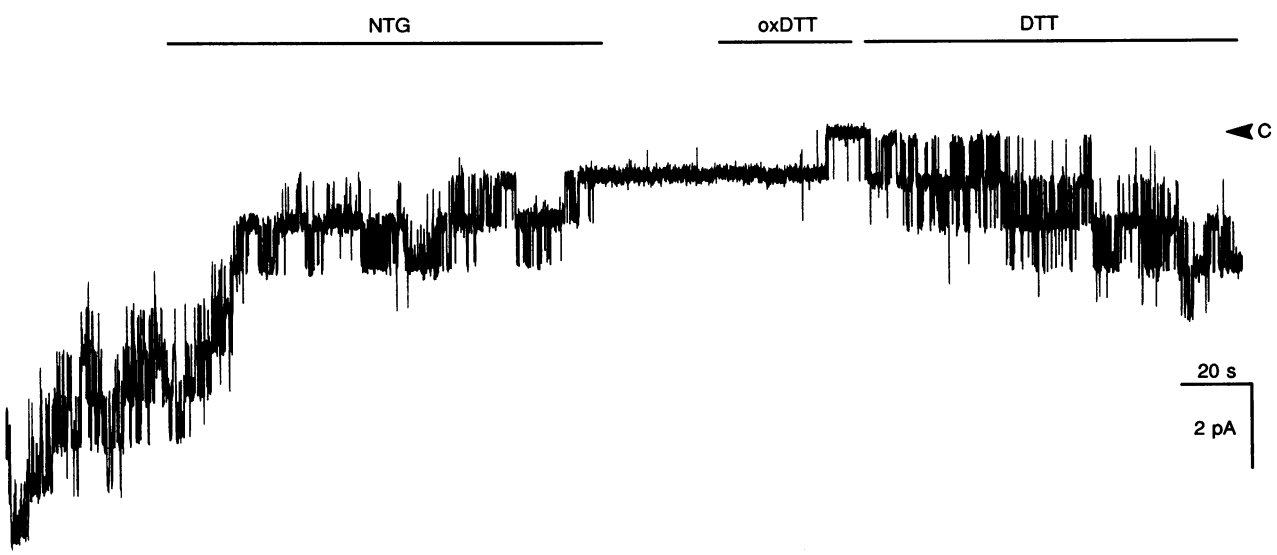

Figure 1. Nitroglycerin-induced blockade of the non-selective cation channel

The membrane potential was −40 mV. Exposure to 100 μM nitroglycerin (NTG), 2 mM oxidized dithiothreitol (oxDTT) and 2 mM dithiothreitol (DTT) is indicated by the horizontal lines. C denotes zero-current level.

27 pS, a linear $I-V$ relationship (−80 to + 80 mV), a slight increase in P_o in depolarizing potentials and a reversal potential of 0 mV in KCl and Ringer solutions, as well as in Ringer solution in which most of the Cl^- is replaced.

All cells used for the present experiments had multilocular fat droplets and were thus mature, fully differentiated cells. In the cell-attached configuration, the pipette potential was clamped to +40 or −40 mV, and no calcium-activated non-selective channel activity was observed. The patch was then excised into Ringer solution. Immediately after excision, the calcium-activated non-selective channel activity became apparent. In all experiments, the Ringer solution was then washed out and high-KCl solution was perfused for ~1 min before application of the test agents. A few patches showed quick run-down during the perfusion and were discarded. However, in most patches, the calcium-activated non-selective cation channels did not run down in the maintained presence of high calcium, and channel activity could be recorded as long as the patch lasted (3–15 min). In the present study, the number of calcium-activated non-selective cation channels per patch varied from two to twelve.

To study the effect of nitric oxide on the putative redox-modulatory site on the calcium-activated non-selective cation channel, we used four agents which are known to release nitric oxide. We found it necessary to use several different agents, as the potential influence of the breakdown products cannot be eliminated in each case. We investigated the effect of two clinically used nitrovasodilators (nitroglycerin and nitroprusside) that generate and release nitric oxide by different mechanisms. These substances may, however, have some other effects (Lei *et al.* 1992; Harrison & Bates, 1993). To facilitate the interpretation, the effects of two more specific agents for generation of nitric oxide (*S*-nitrosocysteine and *S*-nitroso-*N*-acetyl-D,L-penicillamine) were also investigated. In each case, the reducing agent dithiothreitol was subsequently added in excess to regenerate the sulfhydryl groups presumably involved in the redox-modulatory site. As a negative control for reduced dithiothreitol, we used oxidized dithiothreitol.

Effects of nitroglycerin

Nitroglycerin (100 μM) almost completely blocked the activity of the non-selective cation channel (Fig. 1). Although the time for complete perfusion solution change was less than 1 s, it often took several seconds to obtain the full effect. In the control state, the mean open time probability, P_o, was 0·58 ± 0·06 ($n = 5$). After the application of nitroglycerin, P_o was significantly decreased to 0·06 ± 0·02, ($P < 0·001$). The blocking action could not be washed away with perfusion solution alone (30 s application). Further, 2 mM oxidized DTT was not able to reverse the blockade (30 s application) (P_o was 0·04 ± 0·02; $P = 0·91$). However, 2 mM DTT was able to reverse the blockade to a significant extent (P_o was 0·34 ± 0·06, $P < 0·05$).

Effects of sodium nitroprusside

Sodium nitroprusside (100 μM) also potently blocked channel activity (Fig. 2). P_o was 0·74 ± 0·06 ($n = 5$) in the control state; after the application of sodium nitroprusside, P_o was decreased significantly to 0·05 ± 0·03 ($P < 0·001$). As in the situation above, 2 mM oxidized DTT was not able to reverse the blockade (P_o was 0·05 ± 0·03; $P = 0·63$), whereas DTT at a concentration of 2 mM could significantly restore activity (P_o was 0·34 ± 0·11; $P < 0·01$).

It may be noted that nitric oxide is spontaneously released from nitroprusside in the presence of light; this is potentiated by a reducing environment. Cyanide and Fe^{2+} are also released, and these substances are available to react directly with free thiols (Harrison & Bates, 1993). This may complicate the interpretation of the nitroprusside results as such.

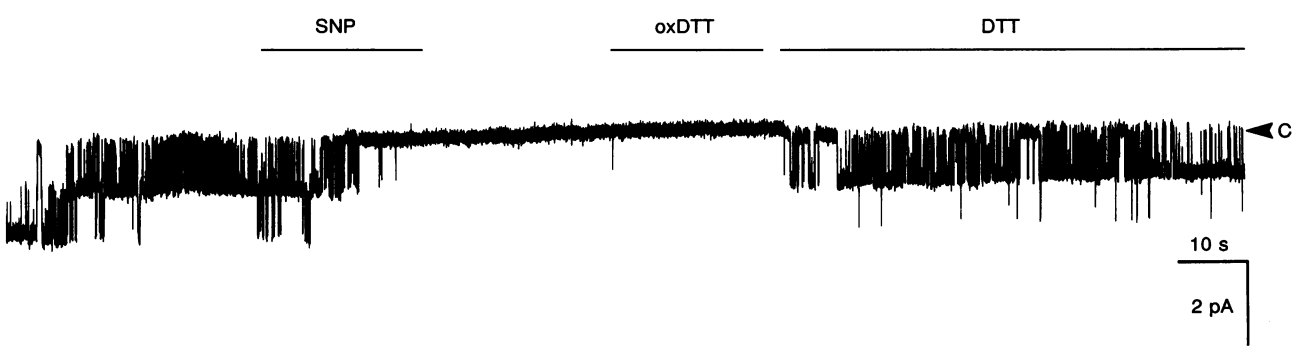

Figure 2. Nitroprusside-induced blockade of the non-selective cation channel

The membrane potential was −40 mV. Exposure to 100 μM nitroprusside (SNP), 2 mM oxidized dithiothreitol (oxDTT) and 2 mM dithiothreitol (DTT) is indicated by the horizontal lines. C denotes zero-current level.

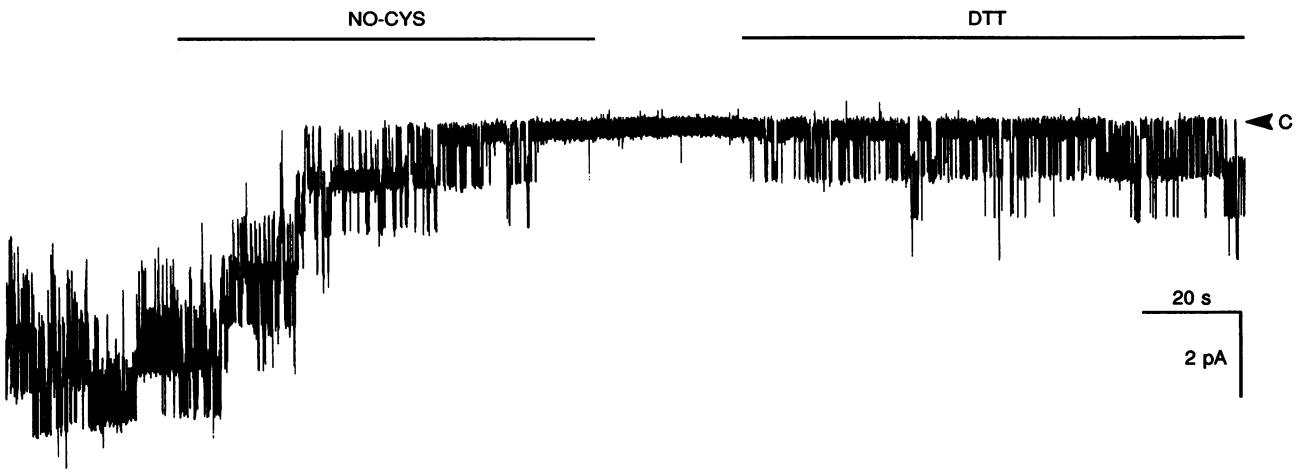

Figure 3. *S*-Nitrosocysteine-induced blockade of the non-selective cation channel
The membrane potential was −40 mV. Exposure to 100 μM *S*-nitrosocysteine (NO-CYS) and 2 mM dithiothreitol (DTT) is indicated by the horizontal lines. C denotes zero-current level.

Effects of *S*-nitrosocysteine

S-Nitrosocysteine (100 μM) also potently blocked non-selective cation channel activity (Fig. 3). P_o in the control state was 0·79 ± 0·06 ($n = 5$), but after the application of 100 μM *S*-nitrosocysteine, P_o was significantly decreased to 0·04 ± 0·03 ($P < 0·001$). We found that the potency of the *S*-nitrosocysteine solution changed with time, very fresh solutions being the most potent. This was probably due to nitric oxide depletion. In some experiments, we tried to avoid nitric oxide depletion by bubbling the perfusion solution with helium, in order to deplete the solution of oxygen. We did not, however, see any differences in channel activity between oxygen-depleted and oxygen-containing solutions: P_o was 0·79 ± 0·07 ($n = 3$) in the control state and during helium perfusion it was 0·74 ± 0·07 ($P = 0·59$).

In a series of control experiments, the *S*-nitrosocysteine solution was kept for more than 6 h at room temperature in order to deplete the solution of nitric oxide. Although the resulting disulphide, cystine, would still be present in the perfusion solution, this solution was now without effect on channel activity: P_o was 0·70 ± 0·16 ($n = 3$) in the control state and 0·70 ± 0·17 under cystine perfusion ($P = 0·99$, not shown). L-Cysteine (100 μM) was also tested and perfusion for 2–4 min was found to be without effect: P_o in the control state was 0·45 ± 0·10 ($n = 3$), and during

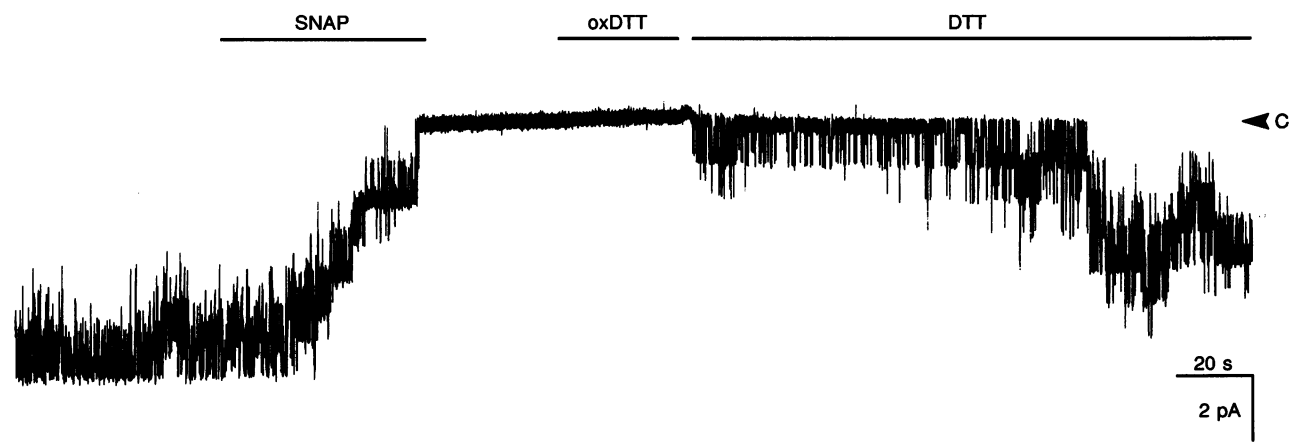

Figure 4. SNAP-induced blockade of the non-selective cation channel
The membrane potential was −40 mV. Exposure to 100 μM *S*-nitroso-*N*-acetyl-D,L-penicillamine (SNAP), 2 mM oxidized dithiothreitol (oxDTT) and 2 mM dithiothreitol (DTT) is indicated by horizontal lines. C denotes zero-current level.

L-cysteine perfusion was 0·36 ± 0·05 ($P = 0·33$, not shown).

The *S*-nitrosocysteine-induced blockade was not spontaneously released during washout with perfusion solution, but was to a significant ($P < 0·05$) extent reversed by perfusion with 2 mM DTT (P_o was 0·23 ± 0·05; $n = 5$) but not with 2 mM oxidized DTT (P_o was 0·06 ± 0·05; $n = 4$).

Effects of *S*-nitroso-*N*-acetyl-D,L-penicillamine

The stable nitric oxide donor *S*-nitroso-*N*-acetyl-D,L-penicillamine (SNAP, 100 μM) also potently blocked non-selective cation channel activity; P_o in the control state was 0·67 ± 0·07 ($n = 6$), whereas after perfusion with SNAP this value decreased significantly to 0·001 ± 0·0008 ($P < 0·001$, $n = 6$). Washout with perfusion solution alone was unable to reverse the blockade. Perfusion with 2 mM oxidized dithiothreitol was also not able to reverse the blockade significantly (P_o, 0·02 ± 0·01; $n = 6$), but addition of 2 mM dithiothreitol was able to reverse the blockade significantly (P_o, 0·22 ± 0·07; $P < 0·05$, $n = 6$; Fig. 4).

In control experiments, we tested 100 μM *N*-acetyl-D,L-penicillamine (NAP), a parent compound of SNAP lacking the nitric oxide moiety, on non-selective cation channel activity. NAP was perfused for 2–4 min but it was always without effect on non-selective cation channel activity: P_o in control state was 0·71 ± 0·09 ($n = 4$) and during NAP perfusion was 0·65 ± 0·10 ($P = 0·44$, not shown).

Effects on channel amplitude

The blocking action of each of these nitric oxide-generating compounds was without effect on the amplitude of the single channel current: the amplitude in the control state was 1·04 ± 0·02 pA and in the presence of NO donors was 1·05 ± 0·02 pA ($P = 0·41$; $n = 10$; data pooled from different experiments). However, during perfusion with 2 mM DTT the single channel amplitude was about 6% smaller (0·98 ± 0·03 pA), i.e. statistically significantly different from the amplitude in the control state ($P < 0·05$; $n = 10$). We did not observe any differences in the blocking potency of the NO donor compounds at membrane potentials of +40 or −40 mV.

Effect of alkylation of sulfhydryl groups

In some experiments we tried to protect sulfhydryl groups from the action of nitric oxide by alkylation with 2 mM *N*-ethylmaleimide (NEM). However, in all cases, alkylation by NEM in itself led to blockade of the non-selective cation channel activity; P_o was 0·62 ± 0·09 in the control state whereas during NEM perfusion it decreased significantly to 0·004 ± 0·003, ($P < 0·01$, $n = 4$). The effect could not be washed out with Ringer solution. However, this blockade was reversible with 2 mM DTT; P_o was 0·50 ± 0·21 ($n = 4$; Fig. 5). This result is in agreement with our previous results concerning the effects of sulfhydryl-group specific reagents on the non-selective cation channel activity (Koivisto *et al.* 1993). Similar effects of NEM on the activity of K_{ATP} channels (Lee, Ozanne, Hales & Ashford,

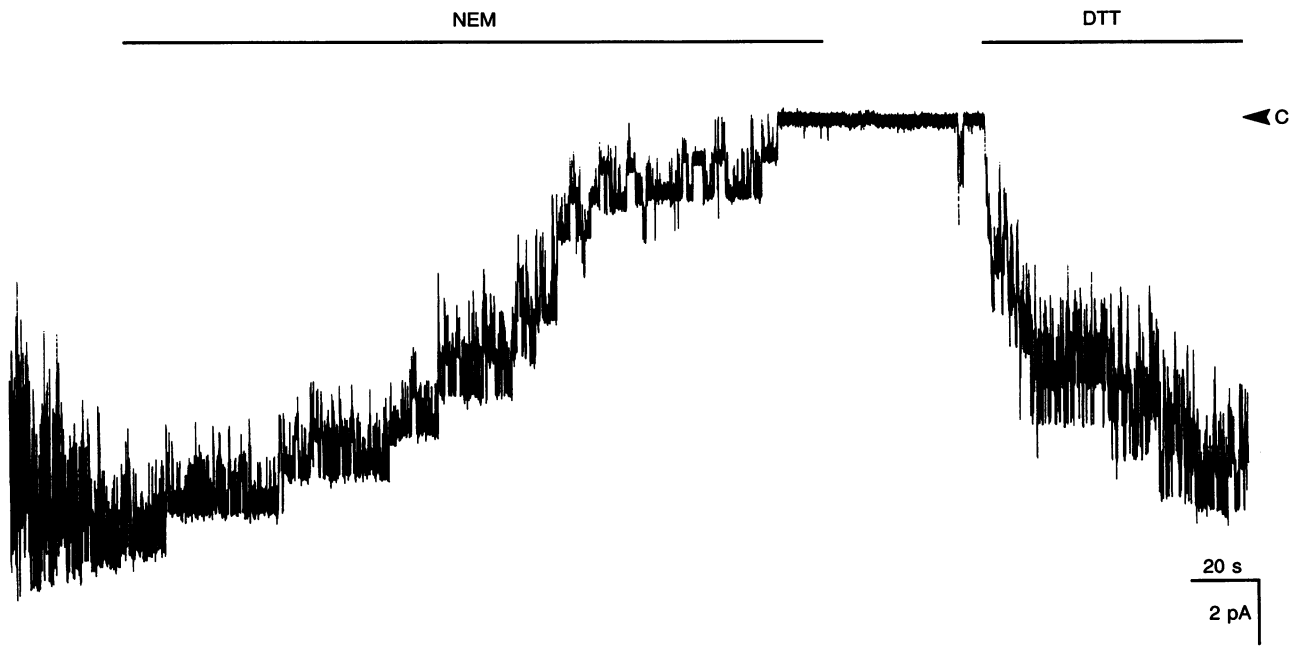

Figure 5. *N*-Ethylmaleimide-induced blockade of the non-selective cation channel
The membrane potential was −40 mV. Exposure to 2 mM *N*-ethylmaleimide (NEM) and 2 mM dithiothreitol (DTT) is indicated by horizontal lines. C denotes zero-current level.

1994) and calcium-activated maxi-K^+ channels (Bolotina, Najibi, Palacino, Pagano & Cohen, 1994) were recently reported.

DISCUSSION

The novel finding of this work is that nitric oxide is able to modulate directly the activity of the calcium-activated non-selective cation channel. The fact that two clinically used nitrovasodilatators, nitroglycerin and sodium nitroprusside, as well as two specific NO-releasing compounds, *S*-nitrosocysteine and *S*-nitroso-*N*-acetyl-D,L-penicillamine, were equipotent in blocking the activity of the non-selective cation channel, suggests a common mechanism of action. As all these agents release nitric oxide and, as all the breakdown products tested were without effect, it is very probable that nitric oxide is the causative agent.

Mode of action of nitric oxide

We have shown earlier that the calcium-activated non-selective cation channel from brown fat cells has highly reactive sulfhydryl groups on the intracellular side of the plasma membrane, and oxidation of these groups leads to channel blockade (Koivisto *et al.* 1993). In the NMDA-receptor channel, nitric oxide has been shown to oxidize sulfhydryl groups of the redox-modulatory site. This action leads to a decrease in the channel activity (Lei *et al.* 1992; Lipton *et al.* 1993). It is likely that this mechanism is also involved in non-selective cation channel blockade. The following observations are in accordance with this interpretation. The blockade induced by nitric oxide was relatively stable and could not be simply washed away by the perfusion solution. The sulfhydryl-regenerating reagent DTT, which in itself had no effect on open time probability (Koivisto *et al.* 1993), was to a large extent able to reverse the nitric oxide-induced blockade, whereas oxidized DTT was without effect. As single nitrosothiols are very labile (Girard & Potier, 1993), the above results would suggest disulphide formation.

The extensive washout period employed before application of the test agents should guarantee that the patch was free from endogenous modulators. As the solutions used in this study did not contain ATP or GTP (the substrates for phosphorylation, cyclic nucleotide production and G protein activation), it is very unlikely that the effects reported here were due to nucleotide-dependent processes.

The concentration of nitric oxide donors used in this study is well above the range of nitric oxide actually measured under physiological circumstances (Malinski & Taha, 1992). However, the time from preparation of the solutions to recording could vary by several minutes, and the fact that nitric oxide has a very short half-life suggests that the actual concentration of nitric oxide must have been much lower than the nitric oxide donor concentration. In this respect it is interesting that SNAP, the most stable NO donor used in this study, was recently measured to generate a stable concentration of about 0·1 μM nitric oxide at 100 μM concentration at 25 °C (Ichimori, Ishida, Fukahori, Nakazawa & Murakami, 1994). This nitric oxide concentration is well within the physiological range. Here it was found that SNAP at this concentration was the most potent of the compounds tested at blocking channel activity.

An important question for further experimentation will be whether nitric oxide blocks non-selective cation channels in the open or closed state.

Taken together, our results extend the hypothesis first formulated for the NMDA-receptor channel (Lei *et al.* 1992; Lipton *et al.* 1993) and recently shown for calcium-activated maxi-K^+ channels (Bolotina *et al.* 1994) that sulfhydryl groups can be part of the redox-modulatory site and that their *S*-nitrosylation by nitric oxide could be a plausible mechanism for reversible ion channel regulation.

Physiological implications in brown fat cells

A full understanding of the role that calcium-activated non-selective cation channels play in the physiology of the brown fat cell has not yet been reached. It is likely that non-selective cation channel activity is the background (Siemen & Reuhl, 1987) for the late and long-lasting depolarization observed after noradrenaline stimulation (Girardier *et al.* 1968; Lucero & Pappone, 1990) and thus may explain the noradrenaline-stimulated Na^+ influx (Connolly, Nånberg & Nedergaard, 1986). As depolarization leads to a decrease in the driving force for calcium and thus to a decrease in the cytosolic Ca^{2+} level (Lee, Nuccitelli & Pappone, 1993), a blockade of the non-selective cation channels by nitric oxide would enable the cell to maintain a high Ca^{2+} level during adrenergic stimulation. In brown adipose tissue, nitric oxide is apparently released during adrenergic stimulation, although it is not known from which cells in the tissue the nitric oxide is released (Nagashima, Ohinata & Kuroshima, 1994); the brown fat cell itself would, of course, be a candidate. In the intact brown fat cells, the degree of channel blockade would depend on the balance between the amount of nitric oxide present and the redox potential of the cytosol. As nitric oxide is produced in a variety of tissues from L-arginine by nitric oxide synthases (Moncada, Palmer & Higgs, 1991; Knowles & Moncada, 1994), and as calcium-activated non-selective cation channels have been demonstrated in several different tissues (Partridge & Swandulla, 1988; Siemen & Hescheler, 1993), including smooth muscle (Wang, Hogg & Large, 1993), our results could have more general significance.

AIZENMAN, E., LIPTON, S. A. & LORING, R. H. (1989). Selective modulation of NMDA responses by reduction and oxidation. *Neuron* **2**, 1257–1263.

BOLOTINA, V. M., NAJIBI, S., PALACINO, J. J., PAGANO, P. J. & COHEN, R. A. (1994). Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. *Nature* **368**, 850–853.

COLQUHOUN, D., NEHER, E., REUTER, H. & STEVENS, C. F. (1981). Inward current channels activated by intracellular Ca in cultured cardiac cells. *Nature* **294**, 752–754.

CONNOLLY, E., NÅNBERG, E. & NEDERGAARD, J. (1986). Norepinephrine-induced Na^+ influx in brown adipocytes is cyclic AMP-mediated. *Journal of Biological Chemistry* **261**, 14377–14385.

GIRARD, P. & POTIER, P. (1993). NO, thiols and disulfides. *FEBS Letters* **320**, 7–8.

GIRARDIER, L., SEYDOUX, J. & CLAUSEN, T. (1968). Membrane potential of brown adipose tissue: a suggested mechanism for the regulation of thermogenesis. *Journal of General Physiology* **52**, 925–939.

HAMILL, O. P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F. J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv* **391**, 85–100.

HARRISON, D. G. & BATES, J. N. (1993). The nitrovasodilators. New ideas about old drugs. *Circulation* **87**, 1461–1467.

ICHIMORI, K., ISHIDA, H., FUKAHORI, M., NAKAZAWA, H. & MURAKAMI, E. (1994). Practical nitric oxide measurement employing a nitric oxide-selective electrode. *Review of Scientific Instruments* **65**, 1–5.

KNOWLES, R. G. & MONCADA, S. (1994). Nitric oxide synthases in mammals. *Biochemical Journal* **298**, 249–258.

KOIVISTO, A., SIEMEN, D. & NEDERGAARD, J. (1993). Reversible blockade of the calcium-activated non-selective cation channel in brown fat cells by the sulfhydryl reagents mercury and thimerosal. *Pflügers Archiv* **425**, 549–551.

LEE, K., OZANNE, S. E., HALES, C. N. & ASHFORD, M. L. J. (1994). Effects of chemical modification of amino and sulfhydryl groups on K_{ATP} channel function and sulfonylurea binding in CRI-G1 insulin-secreting cells. *Journal of Membrane Biology* **139**, 167–181.

LEE, S. C., NUCCITELLI, R. & PAPPONE, P. A. (1993). Adrenergically activated Ca^{2+} increases in brown fat cells: effects of Ca^{2+}, K^+, and K channel block. *American Journal of Physiology* **264**, C217–228.

LEI, S. Z., PAN, Z.-H., AGGARWAL, S. K., CHEN, H.-S. V., HARTMAN, J., SUCHER, N. J. & LIPTON, S. A. (1992). Effect of nitric oxide production on the redox modulatory site of the NMDA receptor–channel complex. *Neuron* **8**, 1087–1099.

LIPTON, S. A., CHOI, Y.-B., PAN, Z.-H., LEI, S. Z., CHEN, H.-S. V., SUCHER, N. J., LOSCALZO, J., SINGEL, D. J. & STAMLER, J. S. (1993). A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. *Nature* **364**, 626–632.

LUCERO, M. T. & PAPPONE, P. A. (1990). Membrane responses to norepinephrine in cultured brown fat cells. *Journal of General Physiology* **95**, 523–544.

MALINSKI, T. & TAHA, Z. (1992). Nitric oxide release from a single cell measured in situ by a porphyrinic-based microsensor. *Nature* **358**, 676–678.

MONCADA, S., PALMER, R. M. J. & HIGGS, E. A. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacological Reviews* **43**, 109–142.

NAGASHIMA, T., OHINATA, H. & KUROSHIMA, A. (1994). Involvement of nitric oxide in noradrenaline-induced increase in blood flow through brown adipose tissue. *Life Sciences* **54**, 17–25.

PARTRIDGE, L. D. & SWANDULLA, D. (1988). Calcium-activated non-specific cation channels. *Trends in Neurosciences* **11**, 69–72.

SIEMEN, D. & HESCHELER, J. (1993). *Non-selective Cation Channels: Pharmacology, Physiology and Biophysics*, ed. SIEMEN, D. & HESCHELER, J. Birkhäuser Verlag, Basel, Switzerland.

SIEMEN, D. & REUHL, T. (1987). Non-selective cationic channel in primary cultured cells of brown adipose tissue. *Pflügers Archiv* **408**, 534–536.

WANG, Q., HOGG, R. C. & LARGE, W. A. (1993). A monovalent ion-selective cation current activated by noradrenaline in smooth muscle cells of rabbit ear artery. *Pflügers Archiv* **423**, 28–33.

Acknowledgements

Financial support was obtained from the Swedish Natural Science Research Council and the Hasselblad Foundation. Technical assistance from Stig Sundelin is gratefully acknowledged. We thank Dr Staffan Johansson for useful comments on the manuscript.

Received 18 April 1994; accepted 5 January 1995.