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ALPHA AND BETA-ADRENERGIC MEDIATION OF MEMBRANE POTENTIAL CHANGES AND METABOLISM IN RAT BROWN ADIPOSE TISSUE

BY L. GIRARDIER AND GISELA SCHNEIDER-PICARD*

From the Department of Physiology, University of Geneva Medical School, 1211 – Geneva 4, Switzerland

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SUMMARY

1. Membrane potential and flavoprotein redox state have been measured simultaneously and continuously in brown adipose tissue in order to determine how nerve stimulation and adrenergic agonists control its metabolic activity.

2. Both trains of nerve impulses and addition of noradrenaline evoke two temporally distinct cell depolarizations. The first rapid depolarization precedes the increase in flavoprotein reduction.

3. With nerve stimulation, at the time of maximum flavoprotein reduction the cell has repolarized or hyperpolarized. The second slow depolarization follows flavoprotein reduction.

4. Phentolamine, an α antagonist, selectively blocks the first depolarization, but not the flavoprotein reduction. However the time of maximum flavoprotein reduction is delayed.

5. Propranolol, a β antagonist, delays the first repolarization until the end of nerve stimulation and inhibits the transient hyperpolarization, second depolarization and flavoprotein reduction.

6. Isoproterenol, a β agonist, or the fatty acid octanoate produce only a transient hyperpolarization and subsequent slow depolarization following flavoprotein reduction.

7. Thus brown adipose tissue contains both α-and β-adrenergic receptors. Stimulation of α receptors produces an early membrane depolarization. Stimulation of β receptors leads to an increase in metabolic activity which then appears to produce slow changes in membrane potential.

INTRODUCTION

Brown adipose tissue (brown fat) is an effector of non-shivering regulatory heat production in small homoeotherms during cold exposure (Smith & Horwitz, 1969). Recently this tissue has also been implicated in the development of obesity in genetically predisposed animals (Trayhurn & James, 1978) and in the regulation of

* To whom correspondence should be addressed.

body weight (Rothwell & Stock, 1979). Its function is mainly controlled by direct sympathetic innervation (Girardier & Seydoux, 1971). The post-synaptic adrenergic receptor, which mediates the metabolic response has been classified as a β-adrenergic receptor (Fain, 1970). Electrophysiological studies have shown that both noradrenaline and sympathetic nerve stimulation depolarize the cells and increase the metabolic activity of brown fat (Horwitz, Horowitz & Smith, 1969; Williams & Matthews, 1974; Seydoux, Constantinidis, Tsacopoulos & Giradier, 1977). However, the temporal relations between the increased metabolic activity and membrane potential changes have not been well defined. Moreover, although both α- and β-adrenergic receptors are involved in the depolarization of brown adipocytes the type of receptor mediating the various components of the response is uncertain (Horwitz *et al.* 1969; Fink & Williams, 1976).

Recently, we developed a spectrometric method which uses changes in the redox state of flavoproteins as an index of metabolic activity (Schneider-Picard & Girardier, 1982). In the present study we have used this method in conjunction with intracellular electrical recording to measure continuously changes in membrane potential and metabolic activity resulting from sympathetic nerve stimulation and the addition of adrenergic agonists.

METHODS

Tissue preparation. Sivz rats (Tierzucht, Zürich), 250–300 g, were used. The interscapular brown fat was quickly removed together with its nerve supply, and superfused in a Perspex chamber at 30 °C, as already described (Seydoux *et al.* 1977). The composition of the Krebs–Ringer solution was the following (mM): Na^+, 143·4; K^+, 5·9; Ca^{2+}, 2·5; Mg^{2+}, 1·2; Cl^-, 128·2; HCO_3^-, 24·9; $H_2PO_4^-$, 1·2; SO_4^{2-}, 1·2; glucose, 5; pH 7·4.

Recording of flavoprotein redox state. The method of recording the flavoprotein redox state was previously described (Schneider-Picard & Girardier, 1982). Briefly, the preparation is illuminated with the excitation wave-length of flavoproteins, i.e. 465 nm. The illumination covered a rectangular area of tissue of about $1{\cdot}2 \times 1{\cdot}8$ mm. This includes $1–2 \times 10^3$ cells. A beamchopper equipped with the appropriate filters allows the measurement of the reflected intensities at the absorption (A_0) and the fluorescence (F_0) wave-lengths of flavoproteins, 465 nm and 540 nm respectively. A_0 and F_0 were recorded continuously on a multichannel pen recorder (WW Electronic Inc., Basel).

Recording of membrane potential. Membrane potentials were measured with conventional 3 M-KCl intracellular electrodes (20–40 MΩ) connected via an Ag–AgCl wire to the input probe of a high impedance amplifier (WPI KS-700, U.S.A.). The input probe was mounted on a piezo stepper (Physik Instrumente, Waldbronn, G) placed on a micromanipulator (Leitz, Wetzlar). The electrode was advanced under microscopic control until its tip touched the tissue. Cells were impaled with one or two 10 μm steps with the piezo stepper (Fromm, Weskamp & Hegel, 1980). Signals were displayed on an oscilloscope (Tektronix) and a pen recorder. The criteria for successful impalements were an abrupt negative voltage deflexion followed in most cases by a slow increase of a few mV. When a stable value was maintained for at least 1 min, the resting membrane potential (V_0) was determined.

Tissue stimulation and drugs. The nerve was stimulated by applying electrical pulses (40 V, 2 ms) between two chlorided silver wires placed on the nerve trunk. Small quantities of noradrenaline (Arterenol, Hoechst AG, Frankfurt) and L-isoproterenol (Sigma, Saint Louis) were injected with a syringe in the superfusion medium close to the region from which the reflected light and membrane potential were recorded. Phentolamine HCl (gift from Ciba, Basel) in aqueous solution was injected in the superfusion medium by means of an infusion pump (Precidor Infors, Basel). DL-propranolol HCl (Sigma, Saint Louis) and sodium octanoate ($C_8H_{15}NaO_2$, Merck, Darmstadt) were added directly to the Krebs–Ringer solution.

Experimental procedure. After equilibration of the tissue in the chamber (for about 30 min) a basal value for reflected light was obtained. To test the integrity of the preparation the nerve was

stimulated at 4 Hz for 4 min and the changes of flavoprotein redox state recorded. When the tissue recovered the membrane potential was recorded. The nerve was again stimulated, and the succeeding variations of both the flavoprotein redox states and membrane potential were recorded. On occasion the membrane potential of a cell could be continuously recorded for more than 60 min. In six experiments recordings were made simultaneously with two intracellular electrodes separated by 300–500 μm during either nerve stimulation or injection of noradrenaline. During the initial depolarization the time course of the recorded potential changes appeared identical (Fig. 1) and the maximum depolarization produced by nerve stimulation was $17 \pm 1{\cdot}5$ mV; the average difference in amplitude recorded with two electrodes was $3{\cdot}4 \pm 0{\cdot}8$ mV. We conclude that the membrane potential was relatively uniform over the region from which the redox states of the flavoproteins were measured. This is consistent with the observation that brown adipocytes are coupled (Revel, Yee & Hudspeth, 1971; Sheridan, 1971) and gap junctions present in appreciable amount (Schneider-Picard, Carpentier & Orci, 1980).

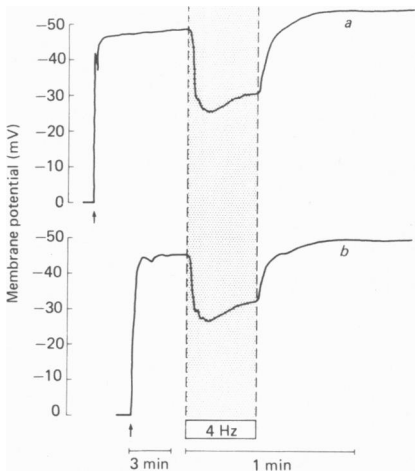

Fig. 1. Simultaneous recording of membrane potential from two cells. The time of penetration is indicated by the arrows. During the period indicated by the stippling the nerve was stimulated by 100 pulses at 4 Hz. At the beginning of stimulation the chart recorder speed was increased by a factor of twelve. The lines *a* and *b* show membrane potential simultaneously recorded from cells a few hundred microns apart. A downward deflexion indicates depolarization.

Presentation of the data. The changes in flavoprotein redox state are measured as fractions of the basal state value (i.e. $\Delta A/A_0$ for changes in absorption, and $\Delta F/F_0$ for changes in fluorescence). Original records were usually accurately traced and then reduced in size with a photocopying machine (Figs. 1, 3, 6 and 7). For Figs. 2 and 8 the record was measured at appropriate intervals and plotted as a continuous line in order to reduce the time axis. From individual records the following parameters have been determined. (1) The amplitudes of maximum reductions for flavoproteins, the amplitudes of maximum depolarizations and the hyperpolarization relative to resting membrane potential, (2) To represent the time course of the variations, we determined the time intervals between start of stimulation and onset of response, between start of stimulation and maximum response and the duration of maximum variations. Means $\pm$ S.E. of means are given. The number of animals used correspond to the number of preparations and are indicated in the Figures. Statistical analysis of the data was carried out with the χ^2 test (confidence level given by P) or the U test of Wilcoxon, Mann & Whitney (confidence level given by α) (Milton, 1964).

RESULTS

Resting membrane potential

The value of measured resting potential (V_0) increased during the course of the experiments from $-45{\cdot}6 \pm 1{\cdot}1$ mV (number of cells = $n_c = 164$; number of preparations = $n_p = 31$) for the first series to $-52{\cdot}3 \pm 1{\cdot}0$ mV ($n_c = 192$, $n_p = 37$, $P < 0{\cdot}003$) in the last series 12 months later. This increase could not be correlated to season, which was shown to influence the activity of the tissue (Girardier, Seydoux, Giacobino & Chinet, 1976), and was most probably explained by improvement of both

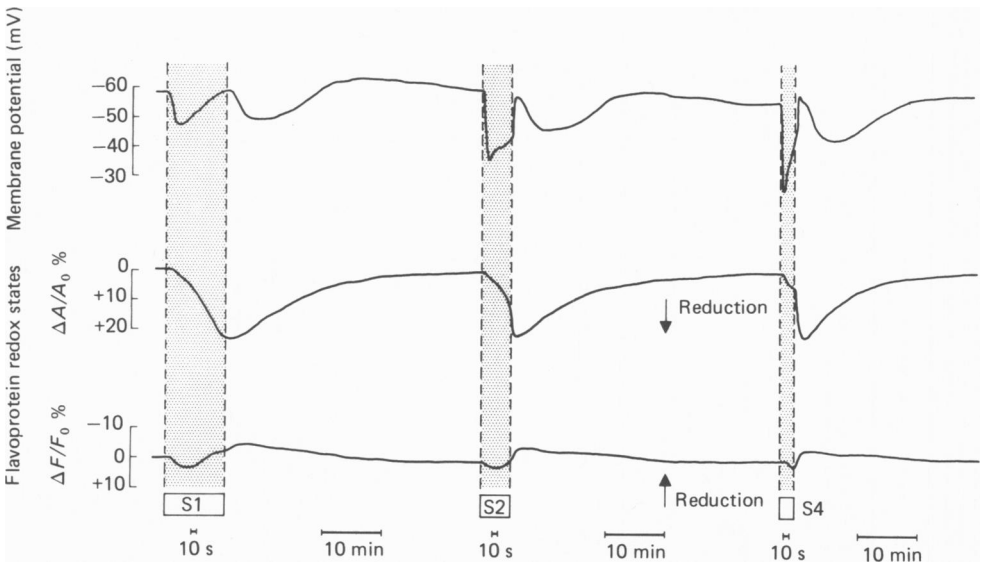

Fig. 2. Variations of membrane potential and flavoprotein redox states evoked by nerve stimulation. During the periods indicated by the stippling, the nerve was stimulated by 100 pulses at 1 Hz (S1), 2 Hz (S2) and 4 Hz (S4). During periods of stimulation the chart recorder speed was increased by a factor of six. The upper line shows membrane potential continuously recorded from the same cell. A downward deflexion indicates depolarization. The middle line shows fractional changes in reflected intensity at 465 nm ($\Delta A/A_0$): a downward deflexion indicates a decrease in absorption i.e. an increase in reduction of flavoproteins (arrow). The lower line shows fractional changes in emitted intensity at 540 nm ($\Delta F/F_0$): an upward deflexion indicates decrease in fluorescence, i.e. an increase in the reduction of flavoproteins (arrow).

tissue preparation and impalements of cells. The abrupt negative voltage deflexion was frequently followed by a slow increase by a few mV during 2–3 min, indicating sealing of the electrode in the cell; it could then fall again slowly, indicating some membrane damage around the site of penetration. For this reason special care has to be taken in evaluating the value of V_0. A stable value for at least 1 min after sealing seemed to be a sufficient criterion. The mean V_0 from all experiments was $-48{\cdot}9 \pm 0{\cdot}3$ mV ($n_c = 556$, $n_p = 103$).

Variations of flavoprotein redox states and membrane potential evoked by nerve stimulation

Fig. 2 shows the effect of nerve stimulations on membrane potential and flavoprotein redox states of brown fat. The cell depolarized in a few seconds and then started to repolarize during stimulation. When stimulation was terminated the cell continued to repolarize, showing transient hyperpolarization for high frequencies ($\geqslant 4$ Hz). The first rapid depolarization was followed by a second slower wave of depolarization, whose time course is in minutes and occurs entirely after stimulation at these train durations.

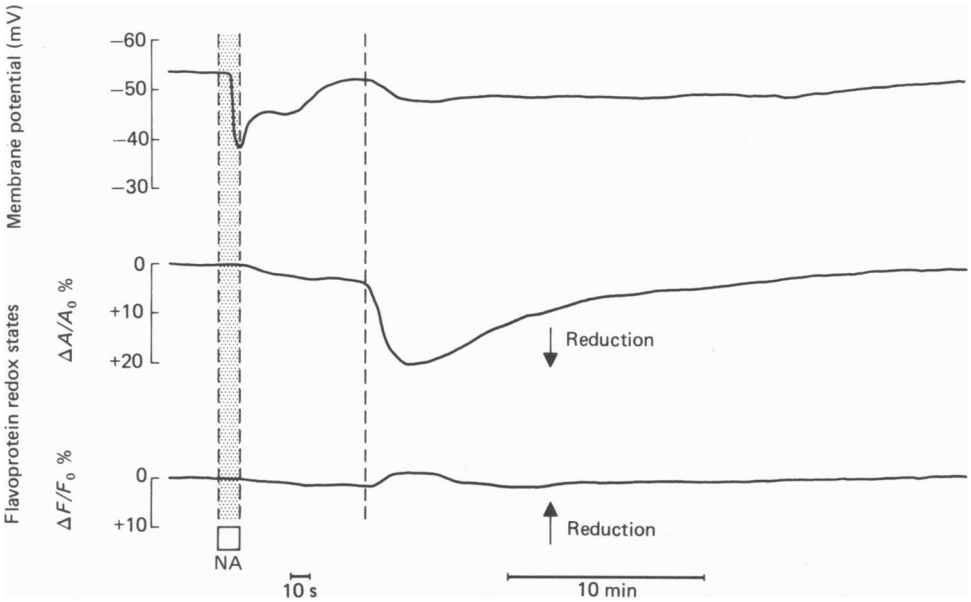

Fig. 3. Variations of membrane potential and flavoprotein redox states evoked by a single injection of noradrenaline (NA). During the period indicated by stippling a small volume of 10^{-5} M-noradrenaline was injected near the recording site. The faster recording speed was used from the beginning of the injection until 80 s after the end of the injection (dashed vertical line). The upper trace shows membrane potential, the middle trace flavoproteins at absorption ($\Delta A/A_0$) and the lower one flavoprotein fluorescence ($\Delta F/F_0$). The arrows indicate increase of reduction.

The tissue absorption (middle trace) decreased, showing a monophasic reduction in flavoproteins related to the first step of β-oxidation (Schneider-Picard & Girardier, 1982). When comparing the time courses of the variations of membrane potential and tissue absorption, it can be seen that the first depolarization occurred when flavoproteins started to reduce, that the cell has repolarized when flavoprotein reduction was maximal, and that the second depolarization occurred when flavoproteins had started to reoxidize.

The tissue fluorescence (lower trace) measuring the redox level of NADH

dehydrogenase (Schneider-Picard & Girardier, 1982), first increased and then decreased, i.e. showing a transient oxidation followed by reduction.

Fig. 3 shows that similar variations in membrane potential and flavoprotein redox states were observed when the tissue was stimulated by exogenous noradrenaline.

Fig. 4 shows the means of the maximum amplitudes and the time courses of the first cell depolarization. The inset of Fig. 4 shows the relation between the maximum

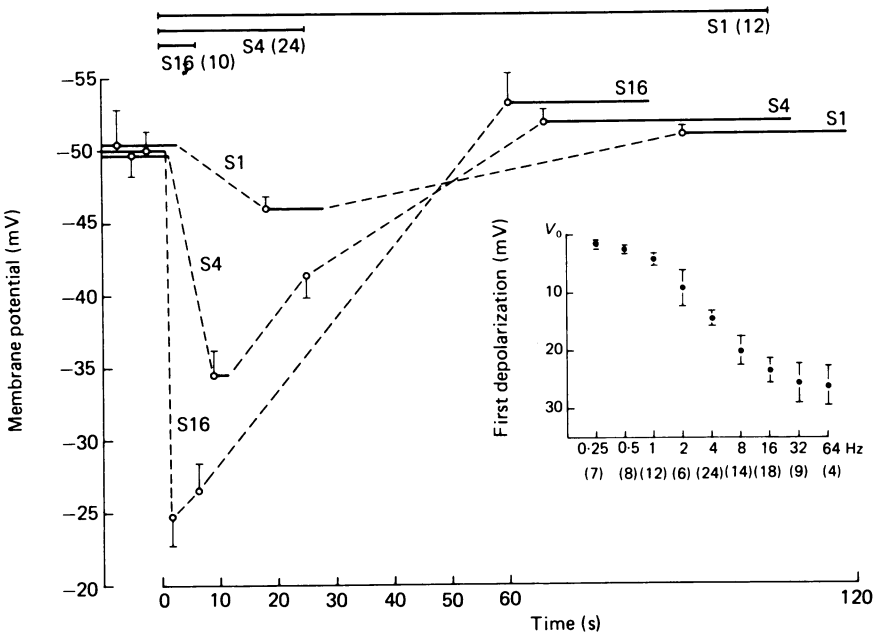

Fig. 4. The variation of membrane potential as a function of stimulation frequency. The lines at the top indicate the duration of nerve stimulation for a train of 100 pulses at 1 Hz (S1), 4 Hz (S4) and 16 Hz (S16). The numbers in parentheses show the number of cells each from a different preparation. The means ± S.E. of means of V_0 and of the maximum amplitudes of the first depolarization and of repolarization are represented. For S4 and S16 the value of the membrane potential at the end of the stimulation is also shown. The continuous lines on the graph indicate the average duration of the maximum variations. The inset shows the relation between the maximum amplitudes of the first depolarization and the stimulation frequency. $\alpha < 0{\cdot}025$ between 0·25 and 1 Hz; $P < 0{\cdot}003$ between 1 and 4 Hz, and 4 and 16 Hz; α not significant between 16 and 32 Hz.

amplitudes of the first depolarization and the stimulation frequency. It can be seen that for a frequency of 0·25 Hz the first depolarization could be detected and that for 32 Hz a maximum depolarization of about 25 mV was reached.

For this series of nerve stimulation by 100 pulses at different frequencies, the maximum amplitudes of the second depolarization (Fig. 2) could be correlated to those of flavoprotein reduction measured at absorption, $r = 0{\cdot}84$, $P < 0{\cdot}01$ (Table 1). As the fluorescence signal was smaller, more complex and more variable we did not attempt to relate it to the changes in membrane potential.

Fig. 5 shows changes in membrane potential and flavoprotein absorption for

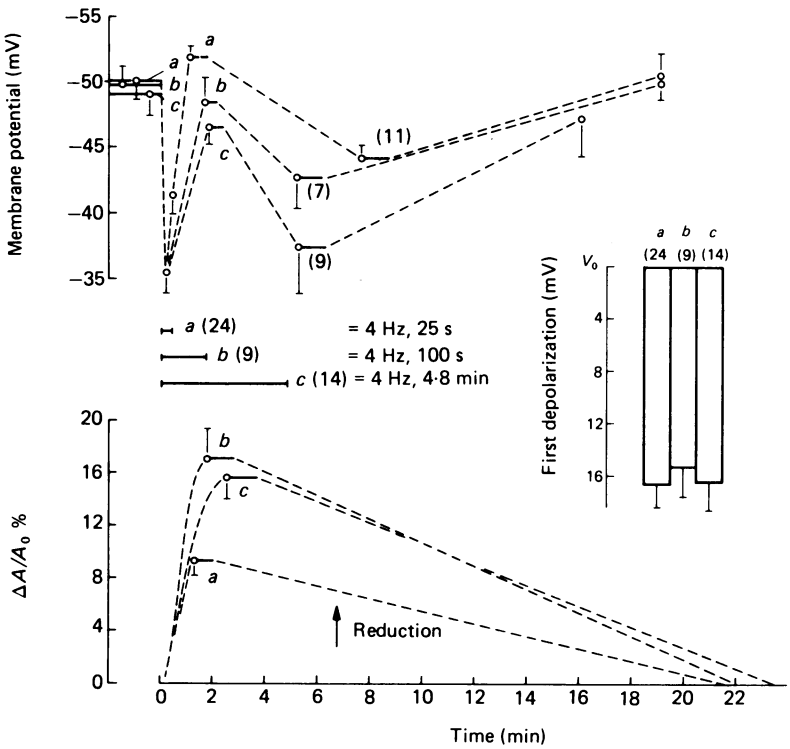

Fig. 5. The variations of membrane potential (upper graph) and flavoprotein absorption ($\Delta A/A_0$, lower graph) as a function of stimulation duration. The duration of nerve stimulation at 4 Hz was 25 s for *a*, 100 s for *b* and 4·8 min for *c*. The means ± S.E. of means of V_0, of the maximum amplitudes of first depolarization, of repolarization, of second depolarization and flavoprotein absorption are shown on the same time scale. To show the time course of the variations, the time intervals have been determined as described in Fig. 4. The smaller number during second depolarization is because some cells were lost during continuous potential recording. The inset shows the maximum amplitudes of the first depolarization for the different stimulation durations.

TABLE 1. Maximum amplitudes of flavoprotein absorption ($\Delta A/A_0$) and of second cell depolarization for nerve stimulation by trains of 100 pulses at different frequencies

Frequency (Hz)	$\Delta A/A_0$) (%)	Second depolarization (mV)
0·25	12·3 ± 5·0 (6)	8·3 ± 1·4 (4)
0·5	20·6 ± 3·9 (4)	10·1 ± 1·4 (4)
1	14·5 ± 2·0 (12)	5·8 ± 1·2 (8)
2	10·1 ± 2·8 (6)	6·4 ± 1·8 (5)
4	9·0 ± 1·0 (24)	4·4 ± 0·9 (14)
8	7·9 ± 1·4 (16)	5·3 ± 1·1 (12)
16	9·8 ± 1·6 (18)	3·4 ± 2·1 (4)
32	6·4 ± 2·0 (5)	3·0 ± 1·8 (5)

Means ± S.E. of means are given. The number in parentheses corresponds to the number of cells each from a different tissue preparation. $r = 0{\cdot}84$, $P < 0{\cdot}01$.

different durations of nerve stimulation at constant frequency. The amplitude of the first depolarization was independent of train duration greater than 25 s (inset). However, the repolarization phase, second depolarization and the amplitude of flavoprotein reduction depended on the duration of stimulation. When the stimulation was terminated during the repolarization phase, transient hyperpolarization (curve *a*) occurred. When the stimulation was continued until maximum reduction of flavoproteins was reached (curves *b* and *d*) in the mean, V_0 was not reached during repolarization. In individual experiments however, transient hyperpolarization was occasionally observed even during prolonged stimulation. The amplitude of the second depolarization increased with increasing train duration.

The changes in membrane potential, especially the first depolarization, and flavoprotein reduction evoked by nerve stimulation, showed more variations between preparations than between successive trials in the same preparation for the first 4 h. After 6 h a decline in responses was observed, and the greatest decline was in the amplitude of the first depolarization. Comparing observations at 120 min with those at 360 min in eight experiments (for 100 stimuli at 4 Hz) indicated that the resting potential, second depolarization and flavoprotein reduction in response to nerve stimulation did not decline significantly. However, the maximum amplitude of first depolarization declined from $16{\cdot}7 \pm 3{\cdot}2$ to $9{\cdot}0 \pm 2{\cdot}0$ mV ($P < 0{\cdot}05$).

The effect of α- and β-adrenergic agonists on the membrane responses and flavoprotein redox state evoked by nerve stimulation

In this series of experiments the addition of a catecholamine antagonist was preceded by a control nerve stimulation of 100 pulses at 4 Hz. At this frequency the first depolarization is easily measured and this number of pulses evoked a large increase in flavoprotein reduction. The train of stimuli was sufficiently short to produce hyperpolarization. When the tissue had recovered from control stimulation, the antagonist was added to the superfusate and its effect on V_0 and on basal flavoprotein redox state recorded. Phentolamine (10^{-5} M), an α antagonist, depolarized the cell from $-46{\cdot}3 \pm 0{\cdot}9$ mV ($n_c = 61$) to $-43{\cdot}4 \pm 0{\cdot}8$ mV ($n_c = 56$), $P < 0{\cdot}02$, and decreased absorption by $2{\cdot}3 \pm 1{\cdot}1$ %, ($n_p = 7$), $P < 0{\cdot}05$.

Fig. 6 shows records of the membrane potential and flavoprotein redox states evoked by nerve stimulation before and after addition of phentolamine. It can be seen (upper trace) that in the presence of the α antagonist, the first depolarization was almost completely blocked, whereas the transient hyperpolarization and the second depolarization, as well as the flavoprotein reduction, were not blocked. Using the same stimulation parameters as in Fig. 6, in seven preparations phentolamine decreased the first depolarization by 95% ($P < 0{\cdot}001$), whereas the amplitudes of hyperpolarization and second depolarization were not affected ($P > 0{\cdot}2$). Although the amplitude of flavoprotein reduction was not affected by the presence of phentolamine, the time interval between onset and maximum of response increased from $1{\cdot}6 \pm 0{\cdot}1$ min in control to $2{\cdot}8 \pm 0{\cdot}4$ min in the presence of the drug ($P < 0{\cdot}005$). In other experiments, it was found, that the effect of phentolamine was dose-dependent. 10^{-6} M-phentolamine decreased the amplitude of the first depolarization by 63%, whereas 10^{-5} M-phentolamine decreased it by 96% ($n_p = 4$, $P < 0{\cdot}002$).

To study the effects of a β antagonist, the same experimental procedure was used.

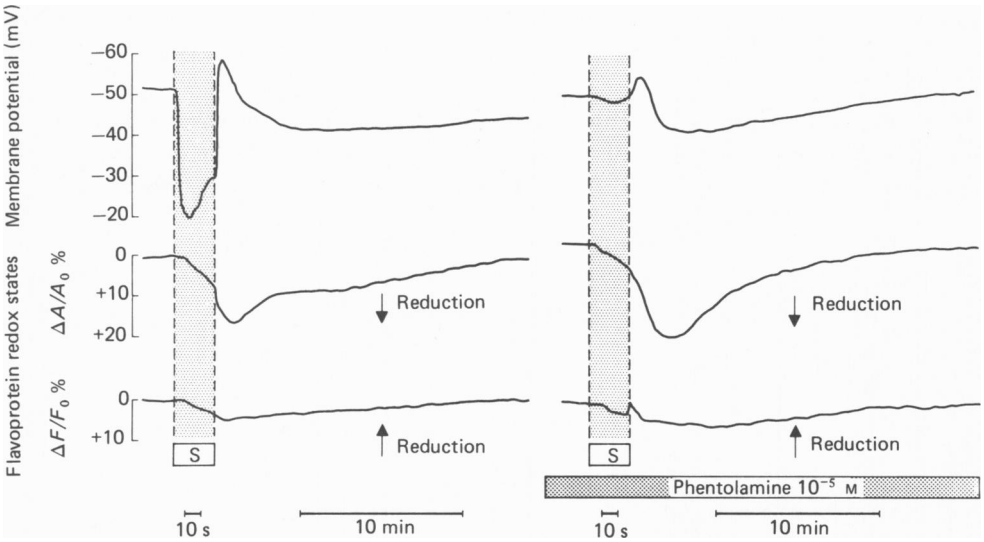

Fig. 6. The effect of phentolamine on the variations of membrane potential and flavoprotein redox states evoked by nerve stimulation. During the periods indicated by S the nerve was stimulated by 100 pulses at 4 Hz. Note the increase in chart recorder speed during stimulation. Phentolamine (10^{-5} M) was added about 30 min prior to the second period of nerve stimulation.

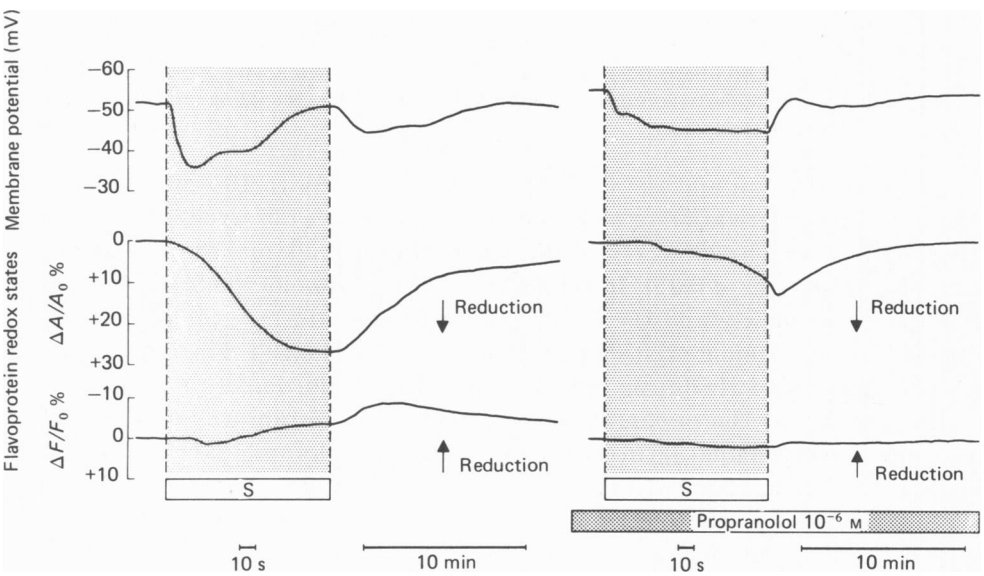

Fig. 7. The effect of propranolol on the variations of membrane potential and flavoprotein redox states evoked by prolonged nerve stimulation. During the period indicated by S, the nerve was stimulated at 4 Hz for 100 s. Note the increase in chart recorder speed during stimulation. Propranolol (10^{-6} M) was added about 40 min prior to the second period of nerve stimulation.

With the addition of 10^{-8} M-propranolol, the basal flavoprotein absorption shifted to a more oxidized state by $2{\cdot}1 \pm 0{\cdot}4$ % ($n_p = 7$), $P < 0{\cdot}005$, and the resting membrane potential decreased from $-49{\cdot}3 \pm 0{\cdot}7$ mV ($n_c = 46$) to $-52{\cdot}7 \pm 0{\cdot}9$ mV ($n_c = 35$), $P < 0{\cdot}005$.

Using the same stimulation parameters as in Fig. 6, in six preparations, increasing concentrations of propanolol (10^{-8}, 10^{-7} and 10^{-6} M) decreased the amplitudes of the measured parameters in a dose-dependent manner; the greatest effect was on the

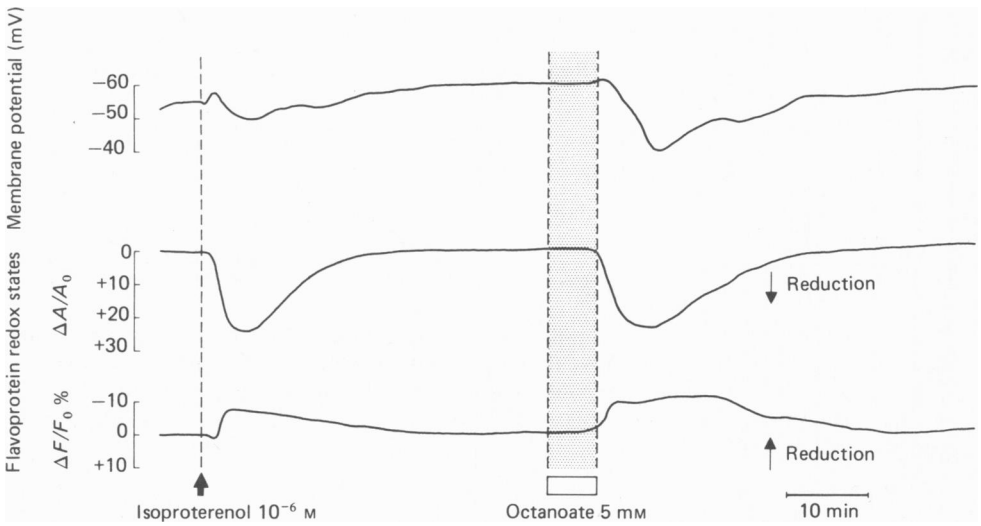

Fig. 8. The variations of membrane potential and flavoprotein redox states after addition of isoproterenol and octanoate. During the period indicated by the arrow a small volume of isoproterenol (10^{-6} M) was injected near the recording site. Octanoate (5 mM) was added to the superfusion during the period indicated by stippling.

second depolarization and the flavoprotein reduction. With 10^{-6} M-propranolol, no transient hyperpolarization or second depolarization was observed; the flavoprotein reduction decreased by 90 % ($\alpha < 0{\cdot}005$), while the amplitude of the first depolarization decreased by 40 % ($\alpha < 0{\cdot}01$). In the presence of propranolol the first depolarization developed more slowly and the cell did not repolarize during stimulation. Thus, in some further experiments the stimulus trains were prolonged. Fig. 7 shows a record for a stimulation at 4 Hz during 100 s before and after addition of 10^{-6} M-propranolol. It can be seen that the maximum of the first depolarization was reached at about 50 s after onset of stimulation in the presence of propranolol and that the repolarization did not occur during stimulation. Using the same stimulation parameters as in Fig. 7, in five preparations, the maximum amplitude of the first depolarization showed a small insignificant decrease but that of flavoprotein absorption decreased by 65 % ($P < 0{\cdot}02$). The maximum amplitude of the second depolarization was decreased by 75 %.

Fig. 8 shows the effects of the addition of isoproterenol, a β agonist, and octanoate, a fatty acid, known to be oxidized by brown fat (Prusiner, Cannon & Lindberg, 1970), on the membrane potential and flavoprotein redox state. It can be seen that reduction

of flavoproteins was accompanied in both cases by transient hyperpolarization, followed by slow depolarization of the cell. In four experiments it was found that 10^{-7} or 10^{-6} M-propranolol did not block the effect of octanoate.

DISCUSSION

We have recorded two temporally distinct depolarizations in response to nerve stimulation or exogenous noradrenaline. The reduction of flavoproteins observed at the absorption wave-length reflects an increase in metabolic activity of the tissue, as this signal is due to changes in the redox state of the mitochondrial flavoproteins implicated in the first step of β-oxidation of fatty acids (Schneider-Picard & Girardier, 1982).

Since the rapid depolarization during the first 20 s of nerve stimulation precedes the increase of flavoprotein reduction it is not secondary to metabolic events occurring after cyclic AMP activation (Reed & Fain, 1968). Furthermore, as the maximum amplitude of this depolarization depends on impulse frequency and not on train duration in the range studied, it is not simply related to the amplitude of the subsequent flavoprotein reduction. The latter depends on both stimulation frequency and duration, indicating some intracellular integration process. An indication of a possible participation of the first depolarization in the metabolic response is that when it is almost completely blocked by phentolamine the maximum reduction of flavoproteins is reached more slowly.

Since hyperpolarization and the second depolarization (produced either by nerve stimulation, a β agonist or octanoate) accompany flavoprotein reduction, these potential changes appear to be secondary to the metabolic changes.

The effects of the adrenergic antagonists on the membrane potential response to nerve stimulation indicate that the first depolarization is mediated by an α-adrenergic receptor and that repolarization or hyperpolarization as well as the second depolarization are mediated via a β-adrenergic receptor. The two depolarizations were observed in all continuous records of membrane potential. Thus, both α and β receptors are located either on the same cell, or since brown adipocytes are coupled (Revel *et al.* 1971; Sheridan, 1971), belong to the same functional syncytium.

The first depolarization is probably secondary to changes in membrane permeability due to direct effect of noradrenaline on the α receptor. The decrease in its amplitude in the presence of the β blocker propranolol cannot, in our opinion, be explained by inhibition of a β receptor stimulation, since isoproterenol, a β-specific agonist, does not produce a rapid depolarization. This inhibition possibly reflects a decline due to duration of the experiments (see Results). A block of nerve conduction by β-antagonists has also been reported (Sada & Ban, 1981).

In contrast to the α effect, the hyperpolarization and the slow second depolarization do not seem to be secondary to changes in membrane permeability due to the binding of the hormone to the receptor, but result from a stimulation of metabolic pathways mediated by the β receptor, possibly those leading to lipolysis via cyclic AMP. This is supported by the observation of similar potential changes after the addition of octanoate, which is oxidized by the tissue (Prusiner *et al.* 1970), and does not stimulate β receptors (see Results).

The small but significant effects of the antagonists on the 'resting' state of the tissue *in vitro*, suggest that there is some base-line liberation of noradrenaline from nerve terminals. This agrees with the observation that the resting potential in brown fat of reserpinized rats is higher than in untreated animals (Fink & Williams, 1976). The stimulation effect of phentolamine could be due to blocking of either pre- or post-synaptic α-adrenergic inhibition (Exton, 1981).

In previous studies, depending on stimulation modes and methods of measurement of membrane potential, either the first or the second depolarization have been observed, but have each been considered as a unique depolarization occurring in brown adipocytes. In the initial observation of brown adipocyte depolarization in response to exogenous noradrenaline (Girardier, Seydoux & Clausen, 1968) only the β-mediated second slow depolarization was observed. Since in this study the potential was measured by successive impalements of different cells, the first depolarization could easily be missed. Thus, these authors could block the observed depolarization by propranolol but not by phentolamine (see also Krishna, Moskowitz, Dempsey & Brodie, 1970).

In studies attempting to correlate electrical and metabolic activity both successive sampling and continuous recording of membrane potential were used. By successive sampling of membrane potential it was shown that cylic AMP, theophylline and octanoate depolarized the adipocytes (Williams & Matthews, 1974). With this method the small transient hyperpolarization could be missed; the depolarizations observed correspond to the second slow depolarization accompanying flavoprotein reduction.

Membrane potential was measured continuously, together with temperature *in vivo* (Horwitz *et al.* 1969) or oxygen consumption *in vitro* (Seydoux *et al.* 1977). The tissue was stimulated by exogenous noradrenaline or nerve stimulation. In both studies depolarization preceded the increase in metabolic activity, and the cells repolarized while temperature and oxygen consumption were still elevated. Thus in both studies it appears, that the first rapid, phentolamine-sensitive, α-mediated depolarization was observed.

Further indication that depolarization of brown adipocytes is mediated by α- and β-adrenergic receptors comes from the study of Fink & Williams (1976), who showed that an α antagonist inhibited α agonist- but not β agonist-mediated depolarization and vice versa. From these agonist and antagonist studies, it was not possible to observe the different time courses of the α- and β-mediated depolarizations, as the potential was measured by successive sampling in different cells. Furthermore, the α-adrenergically mediated depolarization is transient only in the absence of the β antagonist.

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