REDOX TRANSITIONS OF CYTOCHROMES AND PYRIDINE NUCLEOTIDES UPON STIMULATION OF AN ISOLATED RAT GANGLION

B.BRAUSER and Th.BÜCHER
Physiologisch-Chemisches Institut der Universität München, Germany
and
M.DOLIVO
Institut de Physiologie de l'Université de Lausanne, Switzerland

Received 27 April 1970
Original figures received 25 May 1970

1. Introduction

The superior cervical ganglion (0.7 mg wet wt.; 0.3 mm Ø, 2 mm long) of the rat, kept *in vitro* in bicarbonate buffered Krebs solution containing 5 mM glucose, retains its capacity for synaptic transmission and axonal conduction for more than 36 hr [1].

As shown recently, electrophysiological stimulation of the ganglion at frequencies higher than 18 cps results in enhanced oxygen and glucose uptakes of up to 1.5 times the resting rates of 0.3 mmole O_2/g dry wt./hr and 65 μmole glucose/g dry wt./hr. Lactate production also increases by a factor of 2.7 over the resting rate of 40 μmole lactate/g dry wt./hr, indicating stimulation of aerobic glycolysis [1, 2].

Sensitive optical techniques [3, 5] were adapted to the ganglion in order to study the control state of the respiratory chain and the energy supply of the ganglion from the mitochondrial and/or cytosolic compartments during transitions from the resting to the active (stimulated) state.

2. Experimental

2.1. *Preparation of ganglia*

Superior cervical ganglia together with a portion of the pre- and postganglionic nerves, were excised from adult female Wistar rats, 200–300 g body weight, fed on stock diet, as described in [1]. The ganglion was washed free of hemoglobin by perfusion through the a. carotis. The ganglion was positioned in a chamber which was continuously perfused (1 ml/min) with medium consisting of bicarbonate (22 mM) buffered Ringer solution, pH 7.4, containing 5.5 mM glucose. The perfusate was equilibrated with a gas mixture of O_2 and CO_2 (95:5, v/v). The ganglion was kept in a fixed position by moderate tension on the pre- and postganglionic nerve endings. The preganglionic nerve was stimulated by two platinum ring electrodes. The response of the postganglionic nerve was picked up by two similar electrodes, preamplified and displayed on an oscilloscope.

2.2. *Surface fluorometry*

Using the method of Chance and others [3], surface fluorescence of the reduced pyridine nucleotides of the ganglion was excited via a quartz window on top of the perfusion chamber by 366 nm filtered light (interference filter, Netheler and Hinz, Hamburg) from a ST 40 mercury arc lamp.

A 45° illuminator with quartz optics (kindly provided by H.Brück of Leitz GmbH, Wetzlar) and with a 3 mm GG 400 secondary filter (Schott and Gen., Mainz) was used. The detector and its electronics were from an Eppendorf photometer. With this technique a better separation of emitted light from exciting light is obtained than with the Ultropak technique [3] where the excitation beam and the emitted beam are coaxial. The light intensity of the excitation beam was kept low in order to avoid photochemical effects.

2.3. *Dual-wavelength spectrophotometry in transmitted light*

Dual wavelength spectrophotometry in transmitted light was performed with a Rapidspektroskop [4] and a dual-wavelength attachment as previously described [5] (Howaldtswerke Deutsche Werft, Kiel). Parasitic light was excluded by two platinum slits (0.15 × 1.0 mm) which were mounted directly before and after the ganglion. The measuring beam of the Rapidspektroskop (1 × 5 mm) was adapted to the small object by two conical fiber optics (Schott and Gen., Mainz) which piped light into the entrance slit and off the exit slit, thereby condensing the beam to 0.2 × 1.0 mm in the light object plane. It was not possible to use a microscope for this purpose because the height of the perfusion chamber (4 cm) far exceeded the focal length of a microscope.

3. Results and discussion

3.1. *Dual wavelength and fluorometric records during stimulatory cycles*

The ganglion responds to physiological stimulation with an oxidation of respiratory carriers (fig. 1). This reflects the transition from the controlled to the active state. Absorbance of the cytochromes reaches a final value after 40 sec stimulation. It is assumed that this value corresponds to the completely oxidized state of the cytochromes. From the dual-wavelength records at rest, during stimulation, and during anoxia (antimycin A in the case of cyt. *b*), the following degrees of reduction (reduced cyt./total cyt.) were calculated for the resting state: cyt. *b*: 0.53; cyt. *c*: 0.15; and cyt. *a*: < 0.03. This pattern is comparable to the data from controlled isolated liver mitochondria [6] and from perfused liver [7, 8] suggesting a highly controlled state of the respiratory chain within the ganglion.

In fig. 2 the difference trace of (ΔE; 564–575 nm) during stimulation followed by a cycle of anoxia is shown. Interestingly enough, an overshooting reoxidation of cyt. *b* is observed after restoration of the O_2 supply. The magnitude of the overshoot is comparable to the oxidation which occurs during the stimulatory cycle preceding the anoxia. This overshoot may indicate stimulated activity of the respiratory chain due to depletion of the ATP and creatine phosphate pools during anoxia.

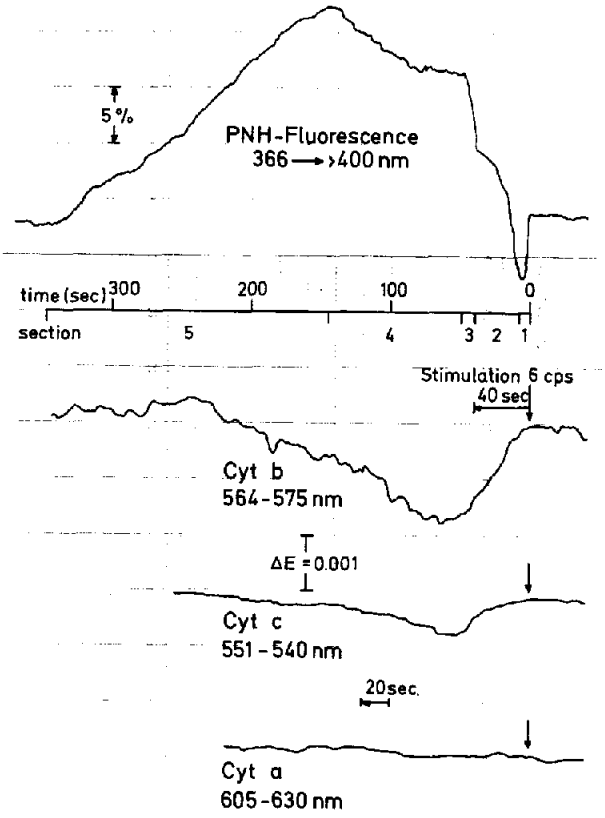

Fig. 1. Redox-transitions by a stimulation period of 40 sec (6 cps; 4.5 V, 1.4 msec square pulses). Downward deflection corresponds to oxidation of the components indicated. Postganglionic response remains constant during all cycles. Longer and/or faster stimulation (not shown) did not produce larger deflections. The linear cytochrome *a* trace indicates sufficient oxygen supply in spite of increased oxygen consumption during stimulation. PNH-fluorescence is scaled in percent of the difference (maximal fluorescence by anoxia, minimal fluorescence by stimulation).

The deflections of the PNH-fluorometric trace are more complex than those of the cytochrome traces. Here, 5 sections may be distinguished during stimulation and subsequent recovery (fig. 1, upper trace): The first event on stimulation is a decrease (section 1, 0–5 sec). It is followed by a rise above the resting level (section 2, 5 sec until end of stimulation). The recovery period starts with a further rapid rise (section 3, 40–50 sec) followed by a slower increase (section 4, 50–150 sec) and a final decrease (section 5,

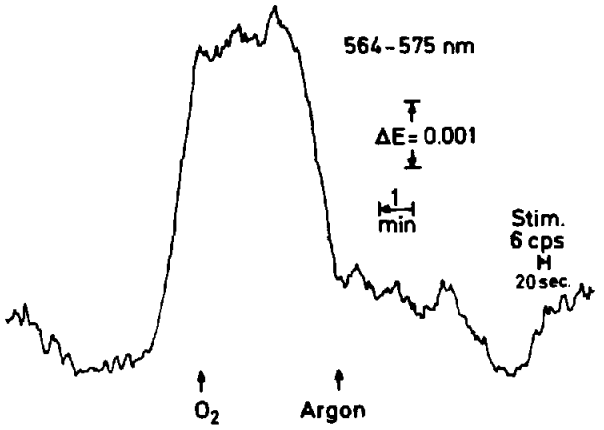

Fig. 2. Record of ΔE (564–575 nm) during a 20 sec stimulation (6 cps) followed by a cycle of anoxia. In addition to cyt. *b*, residual hemoglobin also contributes to the increment during anoxia. Under normoxic conditions where pO_2 stays constant the hemoglobin influence is negligible.

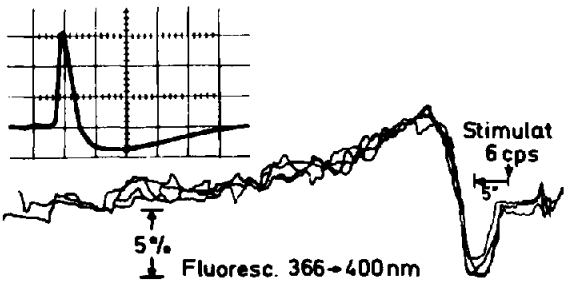

Fig. 3. Superimposed records (right to left) of 5 sec (6 cps) stimulations. A lag time of about 1 sec is observed between the start of stimulation and the fluorometric response. Coincidence of the beginning of upward deflection with the end of the stimulation is accidental.

Above at left one electrophysiological response during stimulation is shown. The calibration is 0.5 mV per square in height and 20 msec in length. Thus the height of action potential is 1.5 mV and the latency between stimulus artefact (dot) and response is 16 msec.

150–340 sec). If stimulation lasts only 5 sec (fig. 3) sections 2, 3 and 4 are not separated. The longer stimulation lasts, the more these sections differ. It appears that the movements at the beginning of stimulation (section 1) and at the beginning of recovery (section 3) are comparable in kinetics and magnitude but of opposite direction. Hence they may be attributed to a rapidly responding PNH system different from a slower reacting one contributing mainly to sections 2 + 4 + 5. What might result from a separation of sections 1 + 3 from the others is shown in the experiment of fig. 4 where by addition of 10 mM pyruvate the metabolic state is influenced in two ways: (a) the cytosolic system is buffered at a low degree of reduction and

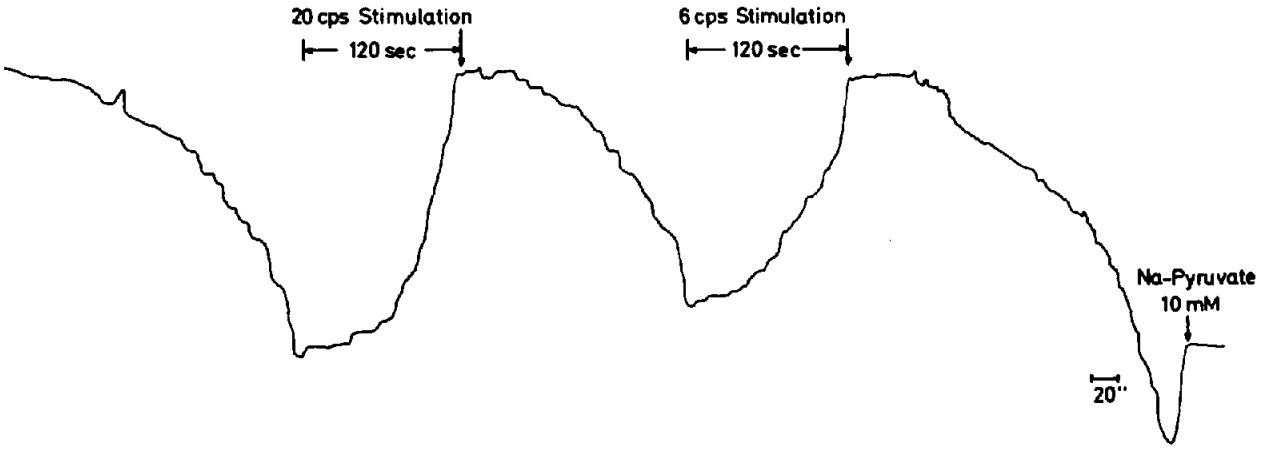

Fig. 4. PNH-fluorometric trace (right to left), demonstrating the effect of (a) addition of 10 mM pyruvate to the glucose containing perfusion fluid and (b, c) the effects of two stimulation-recovery cycles at 6 and 20 cps in the presence of pyruvate. Electrophysiological response was the same as shown in fig. 3 during the experiment.

(b) mitochondrial PNH is saturated. Here stimulation causes only a decrease of fluorescence (section 1). During recovery the fluorescence increases continuously to the initial level (section 3). Therefore sections 1 + 3 may be attributed to the mitochondrial PNH-system, while sections 2 + 4 + 5 reflect cytosolic reactions. Similar results were obtained with 1 mM pyruvate.

3.2. *Function with inhibited respiratory chain*

In a cycle of anoxia postganglionic electrophysiological response decreases to 50% of the control value when PNH fluorescence has reached 90% of its maximum. The same holds when the hydrogen flow is stopped by amobarbital (0.6 mM). Maximal PNH-fluorescence increase produced is the same by amobarbital as by anoxia. The amobarbital concentration for complete inhibition is low (0.6 mM) compared to liver (1 mM). The screening of mitochondria by the microsomal hydroxylating system has been shown for liver [9]; by spectrophotometric measurements [10], cytochrome *P*-450 could hardly be detected in the whole ganglion. This might be a reason for the higher sensitivity to amobarbital. The correlation of fluorescence and function during anoxia or amobarbital inhibition is in agreement with the findings of Terzuolo et al. [11] on the crayfish stretch receptor neuron. However, PNH-fluorescence changes during stimulation and upon substrate addition were not observed with the crayfish neuron in contrast to indirect biochemical assays of PNH [12], whereas such changes were observed with the isolated rat ganglion as described in section 3.1.

References

[1] M.G.Larrabee, P.Horowicz, W.Stekiel and M.Dolivo, Metabolism in relation to function in mammalian sympathetic ganglia, in: Metabolism of the Nervous System, ed. D.Richter (Pergamon Press, London, 1959) p. 36.
[2] M.Dolivo, J. Physiol. 58 (1966) 127.
[3] B.Chance, P.Cohen, F.Jöbsis and B.Schoener, Science 137 (1962) 499.
[4] W.Niesel, D.W.Lübbers, D.Schneewolf, J.Richter and Botticher, Rec. Sci. Instr. 35 (1964) 587.
[5] B.Brauser, Z. Anal. Chem. 237 (1968) 8.
[6] B.Chance and G.R.Williams, J. Biol. Chem. 217 (1955) 409.
[7] B.Brauser and H.Versmold, FEBS Meeting 1968, Prague Abstr. 248.
[8] H.Sies and B.Brauser, in: Inhibitors–Tools in Cell Research, 20th Mosbach Colloquium, eds. Th.Bücher and H.Sies, (Springer, Berlin, Heidelberg, New York 1969) p. 249.
[9] B.Brauser, H.Sies and Th.Bücher, FEBS Letters 2 (1969) 170.
[10] B.Brauser, H.Sies and Th.Bücher, FEBS Letters 2 (1969) 167.
[11] C.A.Terzuolo, B.Chance, E.Handelmann, L.Rossini and P.Schmelzer, Biochim. Biophys. Acta 126 (1966) 361.
[12] E.Giacobini, in: Neurosciences Research, Vol. 1, eds. S. Ehrenpreis and O.Solnitsky (Academic Press, New York, 1968) pp. 1–71.