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SPECTRAL CHANGES IN RESPIRATORY INTERMEDIATES OF BRAIN CORTEX IN RESPONSE TO DEPOLARIZING PULSES*

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SUMMARY

The effect of electrical pulses on the oxidation–reduction states of the respiratory intermediates in isolated rat brain tissue was studied. The results indicate that each of the electron transport intermediates undergoes a transition from a relatively more oxidized to a reduced state. A pulse response spectrum reveals reduced peaks corresponding to NAD(P)H, ubiquinone and cytochromes *c*, *b*, and a–a_3. The amount of cytochrome *c* reduced after 10 sec of electrical pulses is 13 % of the cytochrome *c* reduced by dithionite. These results indicate that the rate of electron transport is dependent on and coupled to adenine nucleotides.

INTRODUCTION

In the previous publication, CUMMINS AND BULL[1] have demonstrated that respiratory intermediates of isolated brain tissue respond rapidly to electrical pulses. The response is biphasic with an oxidative phase lasting only a second, and followed by a rapid reduction in the respiratory intermediates. Since this process is reversible, it was felt that this would be a good experimental system for spectral studies on respiratory responses that may be related to the functional metabolism of excitable tissue. Functional spectral responses have previously been shown by AUBERT *et al.*[2] on the stimulation of the electric organ of *Electrophorus* and by VAN ROSSUM[3] for the effect of methacholine on the avian salt gland. In this publication, we wish to demonstrate that in brain tissue, the entire respiratory chain has a rapid biphasic response resulting in each cytochrome in the respiratory chain becoming relatively more reduced.

METHODS

A detailed description of the manner in which a brain slice is mounted and maintained in the spectrophotometer (Perkin–Elmer Model 356) is given in the previous publication[1]. Electrical pulses (10 V, 0.1 msec) were applied for 15 sec by means of a Grass stimulator. The spectral measurements were similar to that of the

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previous publication. When a spectral response was measured at a number of wavelengths, the reference wavelength was kept constant and balanced with the optical attenuator near the spectral peak. Unless otherwise stated, values are expressed as linear absorbance with 0.003 ΔA equivalent to the distance between the light horizontal lines of the figures.

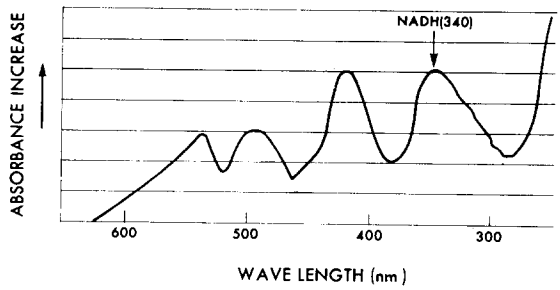

Fig. 1. Absorption spectrum of a brain slice. The spectrum was uncorrected except for subtraction of a spectrum obtained by replacing filter paper for the slice in the chamber. Each horizontal line indicates an absorbance difference of 0.1 unit.

Fig. 1 is an absorption scan of a brain slice reduced with dithionite and measured with both wavelengths at the same setting. The spectrum was corrected by substituting filter paper of similar transmission properties for the slice in the holder. The values obtained for filter paper were subtracted from those obtained for the tissue. This experiment indicates that absorption peaks corresponding to NAD(P)H and the α, β and γ bands of the various cytochromes can readily be measured in brain tissue by the experimental procedures utilized in this publication. The spectral peaks are similar to those obtained from liver and other animal tissues by KEILIN[4,5].

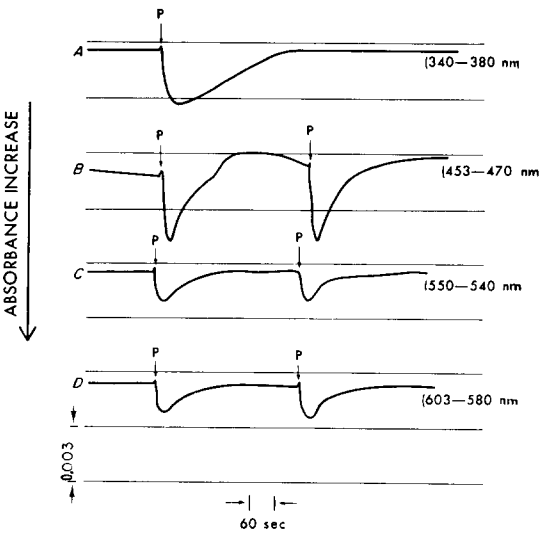

Fig. 2. Effects of electrical pulses at wavelengths corresponding to respiratory intermediates A single slice was used throughout the wavelength combinations A through D.

RESULTS

The pulse response of a single isolated brain slice is repeatable, as shown in Fig. 2, at a number of wavelength selections, and a second pulse at each of these wavelengths gives nearly an identical response. It is interesting that the cytochrome response shows the same biphasic response and recovery as NAD(P)H (Curve A). The wavelength selections which responded to electrical pulses at 550–540 nm corresponding to cytochrome *c*, and at 603–580 nm corresponding to cytochrome a–a_3, indicate that the whole respiratory chain of mitochondria becomes relatively more reduced. Fig. 2 shows that the effects of pulses of short duration are reversible and thus suitable for a number of types of spectral studies such as those that may be related to metabolic control reactions of the tissue.

Fig. 3 represents the response of brain tissue to electrical pulses where the wave lengths have been selected about the reduced absorption peaks of cytochrome a–a_3 (Column A) and of cytochromes *c* and *b* (Column B). Note that Trace A goes from zero response through a maximum response at 603–580 nm and then to another minimum. The same process can be repeated for another slice (Column B) in the cytochrome *c*

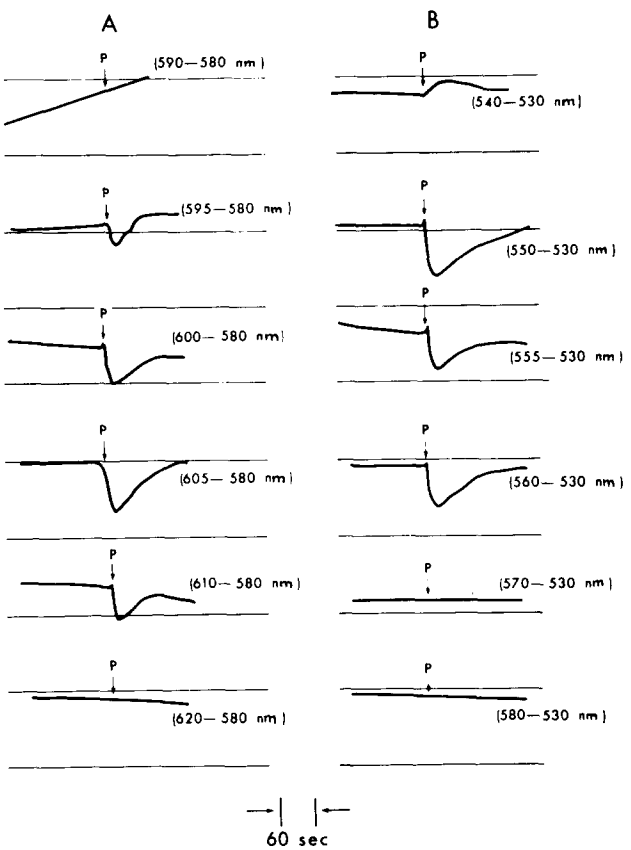

Fig. 3. Response to electrical pulses in the cytochrome a–a_3 region (Column A) and in the cytochrome *b* and *c* region (Column B). Reference wavelength was 580 nm for cytochrome a–a_3 and 530 nm for cytochromes *b* and *c*. One slice was used in Expt. A and another slice used in Expt. B.

and *b* region. This procedure can be performed for other important absorption peaks such as those corresponding to the *γ*-cytochrome region and flavins, to NAD(P)H, and to ubiquinone. A composite of these studies based on the relative response of these regions to electrical pulses is shown in Fig. 4. As one might expect from a measurement based on specific changes in the respiratory components, the peaks are

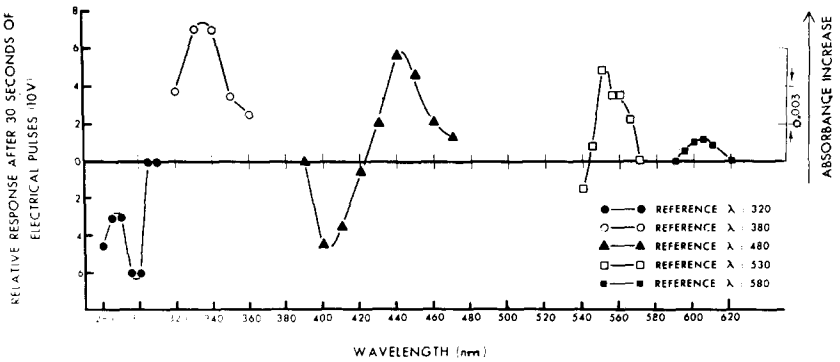

Fig. 4. Spectral response to electrical pulses at wavelength regions corresponding to respiratory changes. Composit curves correspond to: ubiquinone ref. *λ* 320 nm (●—●), NAD(P)H, ref. *λ* 380 nm (○—○), *γ*-cytochromes ref. *λ* 480 nm (▲—▲), cytochromes band, ref. *λ* 530 nm (□—□), and cytochromes *a*–a_3, ref. *λ* 580 nm (■—■).

much sharper than in Fig. 1. Cytochromes *a*–a_3 show a peak at 600 to 605 nm, and it appears that cytochrome *b* at 563 nm can be distinguished from cytochrome *c* at 550 nm. The *γ*-cytochrome region gives a more complex spectrum and shows a shift from oxidized to reduced cytochromes. Possibly there is a flavoprotein component at 460 nm. NAD(P)H shows a sharp peak at 300–340 nm. Below 300 nm there is a small peak that may correspond to ubiquinone. Together, these curves are a significant example of the highly dynamic and coordinate nature of the energy metabolism of cellular systems.

The effect of azide ($5 \cdot 10^{-4}$ *M*) on the cytochrome *c* (500–540 nm) level in isolated brain tissue is shown in Fig. 5. Addition of azide results only in a small perturbation

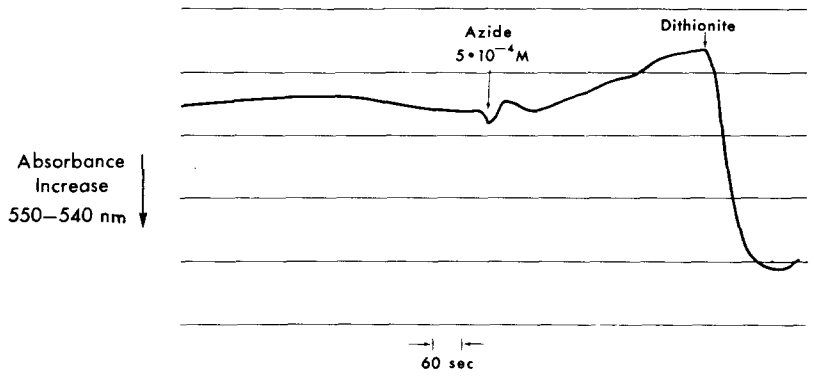

Fig. 5. The effect of sodium azide ($5 \cdot 10^{-4}$ M) and sodium dithionite on the reduced cytochrome *c* level as measured by the difference between 550 and 540 nm.

in the cytochrome absorbance which slowly goes to a more oxidized state. Dithionite dramatically reduces the cytochrome. Table I compares the extent of the dithionite reduction to that obtained with electrical pulses. Although it is not known how much reduced cytochrome *c* is present in the tissue before application of pulses and the extent of reduction of cytochrome *c* by dithionite, it is worthwhile to note that the metabolism increased by electrical pulses is 13 % of the total cytochrome *c* reduced by dithionite. Table I also gives quantitative data on the effect of pulses on the level of NAD(P)H. The standard error is quite reasonable when one considers that the thickness of the slice can vary by 10 %. The extension coefficients are quite different for cytochrome *c* and NAD(P)H, so that these intermediates should not be directly compared.

TABLE I

RELATIVE INCREASE IN NAD(P)H AND REDUCED CYTOCHROME *c* IN RESPONSE TO ELECTRICAL STIMULATION

	Reduced cytochrome c (550–540 nm) (arbitrary units) *	*NAD(P)H (340–380 nm) (arbitrary units)* *
Tissue response	0.60 ± 0.04 (5)	0.88 ± 0.14 (5)
+ Dithionite	4.67 ± 0.78 (4)	—

* 1.0 unit equals an absorbance of 0.003. Number of experiments in parentheses.

DISCUSSION

The spectral response of isolated brain tissues to electrical pulses (Fig. 4) corresponds quite nicely to that previously obtained by KEILIN[4,5] and CHANCE[6] for respiratory intermediates from animal tissues. The method of obtaining the absorption spectra by electrical pulses, and the rapid reversibility of the process, should prove useful for further respiratory studies. A functional cyclic response in the respiratory components was found by CHANCE and co-workers[2,7] in *Electrophorus*, and by LANDOWNE AND RITCHIE[8] in desheathed cervical vagus nerve. In the vagus nerve preparation, the pattern of an initial oxidative phase followed by a more prolonged reduced phase was demonstrated only at physiological temperatures. Direct analysis of the adenine nucleotides and pyridine nucleotides in *Electrophorus* by WILLIAMSON *et al.*[9] showed that NADH decreases after electrical discharge, and rises and falls during the recovery period, a pattern dissimilar to that observed here with brain tissue. The response of brain slices to electrical pulses resembles changes observed by VAN ROSSUM[3] in the avian salt gland where peaks for the reduced cytochromes were induced by ouabain and by methacholine. These various tissue responses seem to reflect the degree of oxidative metabolism in the particular tissue.

It seems clear from the data presented in this and the previous publication that the respiratory system of brain responds within one second to an increased demand of energy. The rapid cycle which is stimulated by electrical pulses to change through relatively more oxidized intermediates to a more reduced state occurs not only in the absorption peaks corresponding to NAD(P)H, but also in cytochromes *b*, *c* and a–a_3.

In addition, the recovery phase is more prolonged for the cytochromes, indicating that the relative reduced state of the cytochromes is longer. This occurs despite the fact that the rates of electron transport consequent to electrical pulses might be expected to be similar to those found for isolated liver mitochondria. CHANCE[10] has given the approximate halftimes of cytochromes *c* and a–a_3 to be of the order of 1–2 msec and for flavoprotein and cytochrome *b* to be about 30–50 msec. More recently CHANCE *et al.*[11] have reported that cytochrome *b* at phosphorylation Site II shifts into an energized phase whose reaction occurs between 200 msec and 2 sec. In our studies, this time closely corresponds to the oxidative phase of the pulse response, and although part of the cytochrome *b* may already be in Phase II, another compartment having to do with the functional response of the nervous system and which is affected by electrical pulses may shift on increased energy demand to a more coupled state (State III). With faster recording equipment, the initial rates of oxidation of the respiratory intermediates of isolated brain tissue may prove to be the same as those obtained with isolated mitochondria.

CUMMINS AND MCILWAIN[12] have estimated that the increased rate of K^+ assimilation due to electrical pulses utilized at least 18 % of the energy of the augmented rate. WHITTAM[13] from studies on ouabain inhibition has estimated that 40 % of the respiration is paced by the active uptake of ions. The effect of the increased energy demand evoked by electrical pulses, as estimated from Table I, is to cause up to 13 % of the cytochrome *c* to become reduced. The previous publication[1] discussed the possibility that adenine nucleotides in the mitochondria may become rate limiting. This is supported by the fact that the rates of cytochrome *c* transit time are of the order of a few milliseconds[5], a rate that should keep the cytochrome oxidized unless it were coupled to ADP + P_i phosphorylation, and which would not account for the increase in reduced cytochrome *c* following pulses. Furthermore, addition of $5 \cdot 10^{-4}$ M azide does not greatly affect the level of reduced cytochrome *c*. These experiments indicate that the energetics of ion reassimilation and other processes evoked by depolarizing pulses are closely linked to reactions involving adenine nucleotides.

The rapidity of the transition evoked by pulses from steady state, to a more oxidized state, and followed by a more reduced state, is indicative of the dynamic processes of cellular metabolism, which involve the interaction between mitochondrial and cytoplasmic components. In *Electrophorus electricus*, electric discharge is accompanied by changes in glycolytic intermediates which are controlled by the activation of phosphofructokinase and phosphorylase *a*; enzymes which control the rate of glycolysis and the levels of the various high energy phosphate intermediates[9,14]. LOWRY *et al.*[15,16] have shown that in brain, glycolysis is controlled by the reaction of adenine nucleotides with phosphofructokinase. LANDOWNE AND RITCHIE[8] suggested from their experiments with vagus nerve, that the increase in NAD(P)H fluorescence is due to an increase in glycolytic flux at the level above fructose diphosphate aldolase. It is probable that the same glycolytic control mechanisms are operating in isolated brain tissue after electrical pulses.

The control of glycolysis and oxidative metabolism should be most important to the specific function of the nervous system. The large contribution of mitochondria to the bioenergetics of the response to electrical pulses might be indicative of intense respiratory activity in synaptic terminals which WHITTAKER[17] believes to account for much of the metabolism of brain slices cut tangentially to the cortical neuropile.

It is worthwhile to note that bovine adrenal chromaffin granules, which are similar morphologically to synaptic vesicles, have been reported to contain a *b*-type cytochrome, flavoprotein(s), and NADH oxidoreductase[18]. The presence of specialized membranes in the synaptic terminals such as synaptic vesicles may contribute metabolic features that might appear unusual when compared to the metabolism of isolated mitochondria.

ACKNOWLEDGEMENT

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REFERENCES

1 J. T. CUMMINS AND R. BULL, *Biochim. Biophys. Acta*, 253 (1971) 29.
2 X. AUBERT, B. CHANCE AND R. D. KEYNES, *Proc. R. Soc. London, Ser. B*, 160 (1964) 211.
3 G. D. V. VAN ROSSUM, *Biochim. Biophys. Acta*, 153 (1968) 124.
4 D. KEILIN, *The History of Cell Respiration and Cytochrome*, Cambridge Univ. Press, Cambridge, 1966.
5 D. KEILIN, *Proc. R. Soc. London, Ser. B*, 98 (1925) 312.
6 B. CHANCE, *Science*, 120 (1954) 767.
7 B. CHANCE, C. LEE AND R. OSHINO, *Biochim. Biophys. Acta*, 88 (1964) 105.
8 D. LANDOWNE AND J. M. RITCHIE, *J. Physiol. London*, 212 (1971) 503.
9 J. R. WILLIAMSON, B. E. HERCZEG, H. S. COLES AND W. Y. CHEANG, *J. Biol. Chem.*, 242 (1967) 5119.
10 B. CHANCE, in B. CHANCE, R. W. ESTABROOK AND J. R. WILLIAMSON, *Control of Energy Metabolism*, Academic Press, New York, 1965.
11 B. CHANCE, D. F. WILSON, P. L. DUTTON AND M. ERECINSKA, *Proc. Natl. Acad. Sci. U.S.*, 66 (1970) 1175.
12 J. T. CUMMINS AND H. MCILWAIN, *Biochem. J.*, 79 (1961) 330.
13 R. WHITTAM, *Biochem. J.*, 82 (1962) 205.
14 J. R. WILLIAMSON, W. Y. CHEANG, H. S. COLES AND B. E. HERCZEG, *J. Biol. Chem.*, 242 (1967) 5112.
15 O. H. LOWRY, J. V. PASSONNEAU, F. V. HASSELBERGER AND D. W. SCHULZ, *J. Biol. Chem.*, 239 (1964) 18.
16 O. H. LOWRY AND J. V. PASSONNEAU, *J. Biol. Chem.*, 239 (1964) 31.
17 V. P. WHITTAKER, *Control Processes in Multicellular Organisms*, Churchill, London, 1970, p. 354.
18 T. FLATMARK, O. TERLAND AND K. B. HELLE, *Biochim. Biophys. Acta*, 226 (1971) 9.