A COMPARISON OF THE INFLUENCE OF 2,4-DINITROPHENOL ON THE OXYGEN CONSUMPTION OF RAT BRAIN SLICES AND HOMOGENATES

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It is well established that the oxygen consumption of various types of intact cells is augmented when low concentrations of 2,4-dinitrophenol (DNP) or certain other substituted phenols are added to the medium in which the cells are suspended. Slightly higher concentrations depress oxygen consumption. This has been shown for yeast (1-6), luminous bacteria (7), various marine eggs (8-14), orthopteran embryos (15), mammalian sperm (16-19), and several amphibian (20) and mammalian (21-25) tissues. Similar observations have been reported in some studies on glycolysis (cf. (26)). The augmentation phase of the concentration-action curves (cf. (27)) which depict the effect of graded concentrations of DNP (and certain other substituted phenols) on oxygen consumption appears to depend upon factors associated with cell structure or rendered inactive by dilution (cf. (28)), because it has not been observed in cytolyzed material (29) or in cell-free preparations of various enzymes directly concerned in cell respiration (13, 30-32). Recently Reiner (33) has described a rat brain homogenate, reinforced with the known co-factors of glycolysis and with nicotinamide, cytochrome c, and fumarate, which is capable of oxidizing glucose. Moreover Reiner has defined the conditions for maximum activity in this system. In order to obtain further information regarding the action of the substituted phenols in augmenting cell respiration, which is still imperfectly understood (26, 34, 35), we have compared the influence of graded concentrations of DNP on the oxygen consumption of rat brain slices and homogenates with glucose as the fuel in each case. The results are presented in this paper.

Methods

Tissue was obtained from twelve adult albino rats of the Slonaker-Wistar strain. After decapitation the brain was rapidly excised and placed in a small chilled beaker standing in a tray of cracked ice. Beaker and tray were transferred to a moist cold box (36). The brain was then removed

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from the beaker and placed on a paraffined stopper. Adherent blood was removed with filter paper and meningeal tissue was stripped off with bone forceps. Cerebral cortex slices were prepared by the Lucite templet method (37) by means of a clean, dry safety razor blade (38, 39). Tissue sections were removed from the cutting blade with fine forceps, and placed in a Petri dish which stood on the surface of a tray of cracked ice. This dish was kept humid by means of a piece of filter paper moistened with Ringer's solution which was stuck on the under surface of the lid. The lid was so placed that the dish was partly open to receive tissue. The slices were arranged in a number of small piles corresponding to the number of flasks to be loaded. When a suitable quantity of tissue was on hand, the dish was closed and the ice tray and dish were removed from the cold box. The slices were rapidly weighed on a micro torsion balance. The usual load placed in a 15 ml. respirometer flask was about 50 mg. of wet weight. Control experiments showed that with sample weights ranging from 20 to 90 mg. respiration varied directly with tissue weight. Aliquot samples (duplicate or triplicate) were placed in small weighing bottles and dried to constant weight in an electric oven at 105°. After loading with tissue, the respirometer flasks, which already contained the necessary solutions (chilled), were attached to the corresponding manometers and oxygenated at room temperature. They were then placed in a water bath maintained at 37.5° ± 0.01°. By this procedure the brain tissue is kept in a cold moist environment from the time of excision until adequate oxygen is available. Thus imbalance between the anaerobic and aerobic aspects of metabolism is minimized (40). The suspension medium was Krebs' Ringer-phosphate, pH 7.35, containing 0.011 M glucose. The gas phase was oxygen. Results are expressed in the conventional Q notation. Thus QO₂ denotes microliters of oxygen consumed, measured under standard conditions, per mg. of dry weight of tissue per hour.

The homogenates were prepared by means of an all glass Potter-Elvehjem (28) homogenizer in ice cold double distilled water. The system for the homogenate work was essentially that of Reiner (33). The following constituents were used in the final (vessel) concentrations given: glucose 0.028 M, hexose diphosphate (HDP) 0.005 M, adenosine triphosphate (ATP) 0.0007 M, coenzyme I (DPN) 0.001 M, potassium fumarate 0.0016 M, nicotinamide 0.04 M, magnesium chloride 0.008 M, cytochrome c 0.00006 M, KH₂PO₄-K₂HPO₄ buffer, pH 7.4, 0.04 M. The HDP, ATP, and DPN were commercial preparations from the Schwarz Laboratories. The former two were supplied as the barium salts and were converted to the potassium salts shortly before use. Potassium fumarate was prepared by neutralizing fumaric acid with potassium hydroxide. Cytochrome c was a commercial preparation (Treemond). All solutions were freshly prepared before each
experiment. Suitable concentrations of 2,4-dinitrophenol (DNP) were made up in Krebs' Ringer-phosphate for the tissue slice experiments and in 0.04 M phosphate buffer, pH 7.4, for the homogenate runs. When used, DNP was added from the side arms of the respirometer flasks 30 minutes after the end of the thermoequilibration period, thus allowing each preparation to serve as its own control for the initial 30 minutes. In all cases steady states of oxygen consumption were observed during the control period. On addition of DNP the maximum effect was very rapidly attained and remained at the same level of intensity for a considerable time. The degree of augmentation or inhibition was calculated from steady state data before and after addition of DNP.

Results

The effect of graded concentrations of DNP on the oxygen consumption of rat cerebral cortex slices in Krebs' Ringer-phosphate solution and of reinforced rat brain homogenate in phosphate buffer at 37.5°C is shown in Fig. 1. An arbitrary value of 1.00 has been assigned to the rate of respiration during the control period (before addition of DNP). It is evident from inspection of Fig. 1, that under the conditions of these experiments the respiration of cerebral cortex slices was augmented in the presence of concentrations of DNP ranging from $4.46 \times 10^{-6}$ to $8.92 \times 10^{-5}$ M and was depressed by concentrations of $1.12 \times 10^{-4}$ M or higher. In the rat brain homogenate the picture was quite different. The concentrations of DNP which augmented respiration in the slices, i.e., $4.46 \times 10^{-6}$ to $8.92 \times 10^{-5}$ M, inhibited oxygen consumption in the homogenate, the degree of inhibition increasing as the concentration of DNP rose. It seemed possible that an augmentation phase in homogenate respiration might occur at lower levels of DNP than in the slice. To see whether this was so we examined the effect of graded concentrations of DNP ranging down to $1.11 \times 10^{-6}$ M. No augmentation phase was found. Concentrations of DNP below $4.46 \times 10^{-6}$ M did not influence homogenate respiration. Thus when oxygen consumption is measured, with glucose as fuelstuff, the concentration-action curve of DNP is diphasic for cerebral cortex slices, which are essentially preparations of intact cells, and monophasic for brain homogenates, in which practically no intact cells were found on microscopic examination (although many apparently undamaged nuclei were present).

There is one interesting feature of the data which is not brought out in Fig. 1 where the values of respiration are given on a relative basis. This feature is the striking similarity between the maximum rate of respiration in cerebral cortex slices in the presence of DNP (in "optimum" concentrations for respiration) and the respiratory rate in homogenates without DNP. Thus the rate of oxygen consumption of cerebral cortex slices in
FIG. 1. Concentration-action curve showing relative oxygen consumption of cerebral cortex slices ( ● ) and reinforced brain homogenates ( ○ ) as a function of the concentration of 2,4-dinitrophenol. An arbitrary value of 1.00 has been assigned to the control rate (no dinitrophenol present). For details see the text.

Fig. 2. Block diagram to illustrate the effect of the concentration of 2,4-dinitrophenol usually evoking maximum increase in the respiration of cerebral cortex slices on the respiration of such slices and of reinforced brain homogenates. Blocks with horizontal and vertical bars represent $Q_{O_2}$ values of cerebral cortex slices and brain homogenates respectively. No dinitrophenol was present in the controls.

the presence of optimum concentrations of DNP corresponded to $Q_{O_2}$ values of 17.5 to 19.9, while in homogenates the $Q_{O_2}$ during the control period (before addition of DNP) ranged from 16.4 to 20.4 with a mean of
17.7 (twenty-six runs). In other words the two systems appeared to have approximately the same respiratory ceiling. This is shown in Fig. 2.

It must be pointed out that the comparison drawn in Fig. 2 is not a simple one. It involves two quite different systems. On the one hand we are dealing with cerebral cortex slices, consisting mostly of intact cells, suspended in an “extracellular phase” medium (cf. (38, 41)), in the presence of optimum concentrations of DNP; on the other hand with a system made up of disintegrated cells, with considerable dilution of cellular material (28), present in an approximation of an “intracellular phase” medium (38, 41) and reinforced with various factors important in glycolysis and respiration. However, in the nature of the case no single medium could be used for this comparison (cf. (38)). It should be noted that the homogenate used was the “complete system” of Reiner (33) in which it appears that the conditions are such as to reveal the full potential respiratory capacity of brain tissue (33). It seems to us that the similarity in respiratory rate between this system and cerebral cortex slices in the presence of optimum concentrations of DNP is more likely to be meaningful than fortuitous.

DISCUSSION

Three major groups of observations bearing on the mechanism of augmentation of cell respiration by DNP are reported in this paper. These are (1) that optimum concentrations of DNP raise the rate of respiration in cerebral cortex slices to about the same level as in brain homogenates without DNP but reinforced to exhibit maximum respiratory activity (with glucose as the fuelstuff in each case); (2) that no augmentation of respiration is obtainable, under the conditions of these experiments, when DNP is added to homogenates; (3) that concentrations of DNP which augment the respiration of cerebral cortex slices produce a slight depression of respiration in homogenates.

On the basis of these observations it is suggested as a tentative working hypothesis that augmentation of cell respiration by DNP is due to inhibition of an enzyme “brake” or regulator (cf. (42) p. 178) by this agent; i.e., that it is a matter of “deinhibition” rather than “stimulation.” In so far as respiration itself is concerned the action of DNP appears to be uniformly inhibitory as shown by its effect on this process in the homogenate. Failure to observe augmentation of respiration in the homogenate treated with DNP may well be due to loss of the regulator by the dilution effect (cf. (28)) during preparation. The high respiratory rate of the untreated homogenate is in consonance with this suggestion.

The view that DNP and substituted phenols of like effect may increase cell respiration by inhibition of a regulator is not new (cf. (25, 30)). How-
ever, this view has been both strengthened and somewhat modified by the evidence presented here. Thus the hypothesis is supported by the observation that the ceiling for respiration is about the same, with glucose as a common fuelstuff, for intact brain cells in the presence of an optimum concentration of DNP and for brain homogenates containing no DNP, prepared so as to exhibit apparently maximum activity. It is modified by the observation that concentrations of DNP which augment respiration in the intact brain cell slightly inhibit respiration in the brain homogenate so that the augmentation phase represents the algebraic sum of the effect of inhibition of the regulator and of the respiratory process rather than simply inhibition of the former. In a tissue in which the latter effect predominates there would be no augmentation phase.

The view that augmentation of tissue respiration by DNP is basically an inhibitory phenomenon introduces unity into the concept of the biological activity of this substance and the substituted phenols having qualitatively similar effects. In general these agents inhibit enzyme activity, and the augmentation of the exergonic (43) processes, respiration and fermentation (cf. (26)), by low concentrations of DNP and the like was a unique and anomalous aspect of their action. Concentrations of the substituted phenols which augment or do not depress cell respiration appear to decrease the energy available for assimilation or work (cf. (34)). Thus such concentrations inhibit division in fertilized Arbacia eggs (8-13), multiplication of yeast (44), and growth and development in the frog (45), in a teleost (46), and in Drosophila (47). They also inhibit assimilation of carbohydrate (48) and of protein (49) in various types of cells, phosphate turnover (49), motility in sperm (16-18), ciliary movement in Arenicola larvae (50), and voluntary activity in the rat (51).

While a fair case can be made for the view that the effect of DNP and substituted phenols of like action is always inhibitory on the enzyme level, the evidence is only presumptive, not conclusive. The hypothesis would be much strengthened if the regulator could be identified, as in the case of the hexokinase reaction studied by Cori and Cori and their associates (52, 53). It might then be possible to depress the respiration of homogenates by addition of the regulator and to relieve this inhibition by addition of DNP. It is planned to deal with these questions in a subsequent investigation.

SUMMARY

1. The oxygen consumption of rat cerebral cortex slices in Krebs' Ringer-glucose solution is augmented when sufficient 2,4-dinitrophenol is added to the medium to give concentrations ranging from $4.46 \times 10^{-6}$ to $8.92 \times 10^{-5}$ M. Higher concentrations of DNP depress respiration.
2. Concentrations of DNP which augment respiration in cerebral cortex slices cause a slight inhibition of oxygen consumption in reinforced brain homogenates. Higher concentrations of DNP cause more marked inhibition. Concentrations of DNP lower than those augmenting respiration in cerebral cortex slices have no effect on homogenate respiration.

3. Concentrations of DNP evoking maximum increase in the respiration of cerebral cortex slices bring the oxygen consumption of the slices to about the same level as in reinforced homogenates untreated with DNP.

4. It is suggested that augmentation of cell respiration by DNP is a matter of "deinhibition" rather than "stimulation," and that the essential feature of the augmentation is inhibition of an enzyme "brake" or regulator.

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