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Hypoxia and the Energy Charge of the Cerebral Adenylate Pool

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A brief period of anoxia *in vivo* causes a transitory decrease in the size of the adenylate pool in the rat brain. This is probably caused by feedback inhibition by AMP of adenine nucleotide synthesis. Exposing rats to various degrees of hypoxia suggests that the sensitivity of the brain to lack of O_2 results from the brain's limited ability to maintain an adequate energy charge in unfavourable circumstances.

Compared with other tissues the brain is very sensitive to lack of O_2. It has long been suspected that this vulnerability arises from a failure of the energy-yielding systems of the brain. To investigate this possibility many workers (e.g. Albaum *et al.*, 1953; Thorn *et al.*, 1958; Döring *et al.*, 1959; Cohen, 1962; Minsker *et al.*, 1970) have therefore examined changes in the cerebral high-energy phosphates after hypoxia or ischaemia. Decreases in ATP and phosphocreatine contents and an increase in ADP and AMP contents were generally found, but only Cohen (1962) was able to point to a biochemical lesion behind the observed changes: slices of cerebral cortex show a decreased ability to synthesize ATP after a period of anoxia *in vitro*. I have looked for a similar effect *in vivo*.

Rats were subjected to a period of anoxia or hypoxia with or without a subsequent recovery period in air. The ATP, ADP, AMP and phosphocreatine concentrations in the brains were measured. This information was examined in relation to the concept of energy-charge regulation of metabolism in the brain under various experimental conditions.

Methods

Wistar albino rats weighing 150–200g were used throughout. They were exposed to mixtures of O_2 and N_2 of known composition in glass cylinders (30cm long, internal diameter 8.5cm) through which the gas mixture was passed at 700ml/min. The composition of the gas mixture was controlled and monitored by regulating the flow of each component into a mixing vessel by means of calibrated flow meters. Thus compositions of all gas mixtures are expressed on a v/v basis. Before entering the animal chambers the gas was moistened by bubbling through water. Flow meters were also placed at the outlets of the animal chambers to ensure that the required flow was maintained through each chamber. Before animals were placed in the chambers the gas mixture was allowed to flow for at least 10min.

After exposure to known gas mixtures for known periods some groups of animals were removed from the chambers and allowed to recover in air for known periods before being killed. In this recovery period no attempt at artificial respiration was made.

The rats were killed by immersion in liquid N_2. The brains were removed in the frozen state and the whole organ was homogenized in $HClO_4$ (0.4M) without being allowed to warm up. AMP was determined by using AMP deaminase (EC 3.5.4.6) (Kalchar, 1947) after conversion of the ADP in the sample into ATP with phosphoenolpyruvate and pyruvate kinase (EC 2.7.1.40). This was necessary because ADP was also a substrate for the deaminase. ADP and ATP were assayed with firefly lantern extract (Strehler, 1968); an Aminco fluoromicrophotometer was adapted for the purpose. Phosphocreatine was measured as P_i by the method of Berenblum & Chain (1938) after precipitation of the P_i and hydrolysis of the phosphocreatine with acid molybdate. Recoveries were: AMP 104%, ADP 78%, ATP 86% and phosphocreatine 76%. All biochemicals were supplied by Sigma Chemical Co., St. Louis, Mo., U.S.A.

Results

The brains of normal rats killed by immersion in liquid N_2 contained (μmol/g fresh wt., mean ± S.E.M., number of animals in parentheses): ATP, 2.01 ± 0.15 (20); ADP, 0.36 ± 0.03 (20); AMP, 0.34 ± 0.04 (20); phosphocreatine, 2.56 ± 0.10 (26). These values were unchanged if the animal breathed either air, O_2 or O_2+N_2 (8.5:91.5) in the chambers for 15min. When the O_2 was decreased to 5% by volume the brain phosphocreatine concentration decreased to a new steady state at about 50% of its normal value, the amount of ATP was also halved and that of ADP more than doubled (Fig. 1). In one experiment in which the O_2 was decreased to 3.5% by volume most of the animals died, but the survival times were very

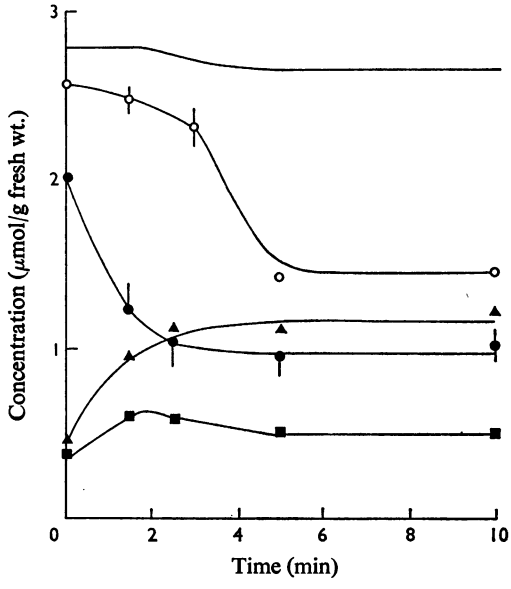

Fig. 1. *Effect of breathing a gas mixture (O_2+N_2, 5:95) on the phosphocreatine, ATP, ADP and AMP concentrations of the rat brain*

Exposure began at zero time and continued for the periods indicated. Full error bars are 2 S.E.M. in length. Each point is a mean derived from at least six animals. o, Phosphocreatine; ●, ATP; ▲, ADP; ■, AMP; ——, total adenine nucleotides (ATP+ADP+AMP).

varied, indicating that this pO_2 was close to the critical value.

In Figs. 2 (solid line) and 3(*a*) are shown respectively the time-courses of the changes in amount of phosphocreatine and the adenine nucleotides in animals exposed to moist O_2-free N_2. The phosphocreatine concentration decreased to a value close to zero in 5min; the concentration of ATP reached a limiting value of 0.25 μmol/g in about the same time, that of ADP fell steadily, and was close to zero in 10min and that of AMP rose to a maximum of 1.55 μmol/g at about 8min and thereafter decreased, presumably as a result of the activity of the brain's AMP deaminase.

When rats were exposed to moist O_2-free N_2 for 30s and then allowed to recover in air, virtually all the animals survived; those that did not were excluded from the results. Only very few animals were able to survive 60s exposure to N_2.

The behaviour of the animals exposed to N_2 followed a stereotyped pattern. There was an initial period of struggling that lasted about 20s. After this the animal was apparently unconscious and respiration had ceased. After a quiescent period of about 10s there was a series of convulsions that lasted about 15s, then about 5s later there was a great, open-mouthed gasp. It was noteworthy that there were no respiratory attempts between the arrest of respiration at 20s and the gasp at 50s. If the 50s gasp took place in the chamber in N_2 the animal did not recover on subsequent removal to air because no further spontaneous respiratory attempts were made. If, however, the 50s gasp took place in air the animal recovered. After the gasp there was a further period of respiratory arrest lasting for about 20s. When respiration recommenced it was initially shallow and irregular, becoming deeper and more regular and normal over about 60–90s. Thus about 2.5min after the beginning of the 30s anoxic period normal respiration was restored. By this time most animals were fully conscious and beginning to right themselves. They were up and exploring by 5min. No long-term recovery experiments were performed and, as far as could be ascertained by superficial observation, recovery was an all-or-none phenomenon.

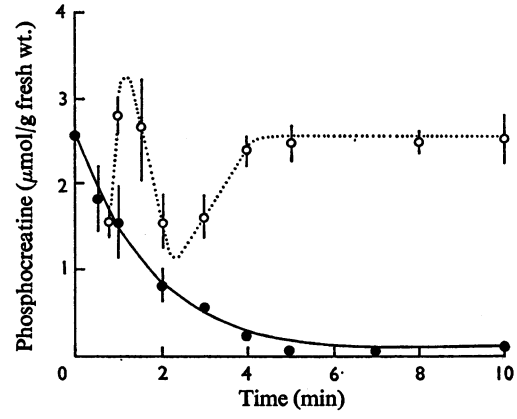

Fig. 2. *Changes in the phosphocreatine content of the rat brain after a period of anoxia in vivo*

——, Animals exposed to anoxia (100% N_2) for the period indicated; ·····, animals exposed to anoxia (100% N_2) for 30s followed by unassisted recovery in air. Each point is the mean of values from at least six animals; the error bars are 2 S.E.M. in length.

The changes in amounts of phosphocreatine and adenine nucleotides during 30s anoxia and recovery are presented in Figs. 2 (dotted line) and 3(*b*) respectively. The amount of phosphocreatine continued to decrease after removal of the animal to air until the 50s gasp, when it rose very rapidly to a value in excess of 3 μmol/g in about 30s. This indicates a net rate of synthesis of phosphocreatine of at least 200 μmol/h per g, which compares with a maximum rate of 150 μmol/h per g quoted by McIlwain (1966). The

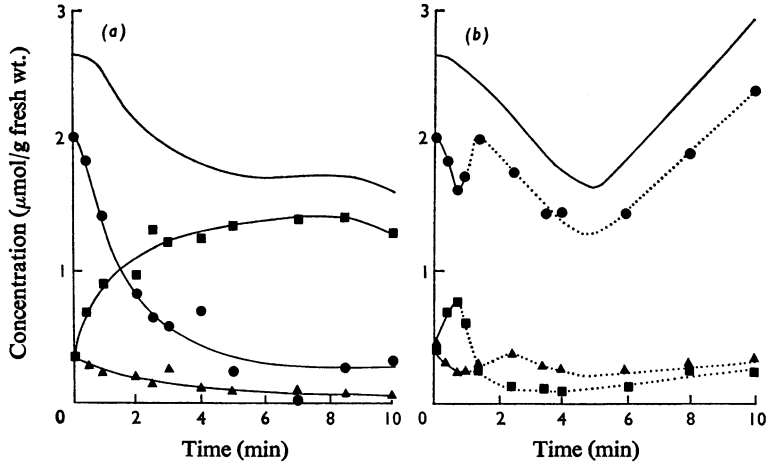

Fig. 3. *Changes in concentration of the adenine nucleotides after a period of anoxia in vivo*

(*a*) Effects of anoxia (100% N_2) for the period indicated. (*b*) Effects of anoxia (100% N_2) for 30s followed by unassisted recovery in air. ●, ATP; ▲, ADP; ■, AMP; ——, total adenine nucleotides (ATP+ADP+AMP). Each point is the mean of values from at least six animals.

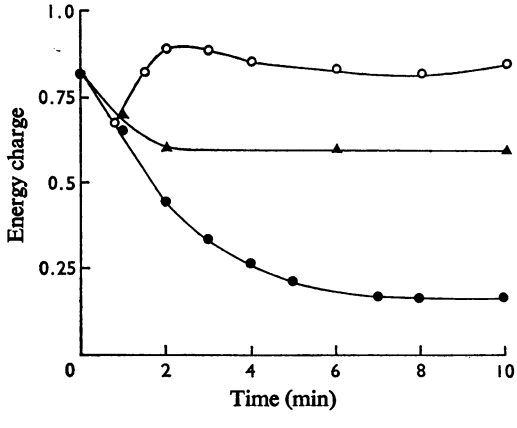

Fig. 4. *Time-course of the changes in the energy charge of the adenylate system of the rat brain*

For experimental details see the text. ●, 100% N_2; ▲, O_2+N_2 (5:95); ○, 100% N_2 for 30s followed by unassisted recovery in air.

peak at 1.25min was followed by a decrease in concentration, which reached a minimum at 2.5min, and a slower steady return to the normal value at 5min.

The amount of ATP also continued to decrease until the 50s gasp, after which it rose to its normal value at 1.5min, a net rate of synthesis of about 30μmol/h per g. There followed a slow decline to a minimum (1.35μmol/g) at 5min and a subsequent rise to an above-normal value at 10min. The AMP concentration decreased rapidly at the 50s gasp and continued to fall very slowly until 4min when it began to increase slowly. The ADP concentration showed no very marked changes.

The energy charge of the adenylate system is defined as half the average number of anhydride-bound phosphate groups per adenine moiety and may assume any value between 1 (only ATP present) and 0 (only AMP present) (Atkinson, 1968). The time-course of this parameter was calculated from the results presented above and is plotted in Fig. 4. The energy charge of the normal rat brain was 0.81, a value very similar to that for other tissues. On exposure to anoxia the energy charge of the brain began to decrease immediately and rapidly, reaching 0.17 after 10min. In the brains of animals subjected to O_2+N_2 (5:95) the energy charge decreased, but a new steady state of 0.60 was established after 2min. In the brains of the rats allowed to recover from 30s of anoxia the energy charge, after an initial decrease, returned rapidly to normal and was maintained there in spite of the changes in total adenine nucleotides shown in Fig. 3(*b*).

Discussion

Synthesis of adenine nucleotides

Figs. 2 and 3(*a*) show clearly the expected decrease in the concentration of brain high-energy phosphates during anoxia. There is also a drastic decrease in the concentration of total adenine nucleotides (Fig. 3*a*).

This probably resulted from a decreased rate of synthesis rather than an increased rate of destruction, as brain AMP deaminase is activated by ATP (Muntz, 1953; Weil-Malherbe & Green, 1955), the concentration of which was very much diminished. A decreased rate of synthesis may *a priori* be related to either an increase in the concentration of AMP, which inhibits purine nucleotide biosynthesis, or to a decrease in the concentration of ATP, which is required in a number of steps in its own synthesis *de novo*. The results favour the idea that the changes are caused by feedback inhibition by AMP. Fig. 1 shows that a steady-state concentration of ATP as low as 50% of normal can maintain a normal total adenylate content. In this case (O_2+N_2, 5:95) the steady-state AMP concentration was only slightly above normal; in anoxia the concentration of AMP increased immediately and rapidly (Fig. 3*a*) and was associated with a correspondingly rapid decrease in the concentration of total adenylate. Fig. 3(*b*) shows that the inhibition was only slowly released after the readmission of O_2, although the AMP concentration decreased immediately.

Energy charge of the adenylate system

The energy-charge concept (Atkinson, 1968) regards the adenine nucleotide system as the energy 'currency' of the cell. Some reactions expend this currency (*U* reactions) and some generate it (*R* reactions). Atkinson (1968) has shown that the rates of *U* reactions increase and the rates of *R* reactions decrease as the energy charge increases (Fig. 5). A steady metabolic state is attained at the energy charge at which the *U* and *R* curves intersect. Any event that changes the energy charge activates homeostatic mechanisms.

An important corollary of the energy-charge concept becomes evident if one particular implicit assumption is stated explicitly. The assumption is that the solid curves in Fig. 5 are individual members of a family of curves whose limiting members are AC and ADC for the *U* reactions and BD and BCD for the *R* reactions. From this it follows that the lowest energy charge for which a steady metabolic state is possible is 0.5. At energy charges of less than 0.5, *U* and *R* reactions cannot be balanced and any situation that prevents the energy charge from reaching or exceeding the critical value must be lethal for the cell. A high energy charge is synonymous with a high degree of phosphorylation of the adenine nucleotides, and since phosphorylation is associated primarily with aerobic *R* reactions hypoxia presents a serious threat to the aerobic cell.

The rat brain withstood a moderate degree of hypoxia. At O_2+N_2 (8.5:91.5) normal concentrations of ATP, ADP, AMP and phosphocreatine were maintained. As the hypoxia was intensified (O_2+N_2, 5:95) the brain could no longer maintain a high energy charge and attained a new steady state corresponding to an energy charge of 0.6 while maintaining the concentration of total adenylate at the normal value (Figs. 1 and 4). Further decrease of the O_2 amount (O_2+N_2, 3.5:96.5) was lethal in most cases and was probably associated with an energy charge close to 0.5. In anoxia there was an immediate and rapid decline in energy charge (Fig. 4). When O_2 was readmitted after 30s of anoxia the energy charge quickly rose to, and was maintained at, the normal value (Fig. 4) despite wide variations in the concentration of total adenine nucleotides (Fig. 3*b*).

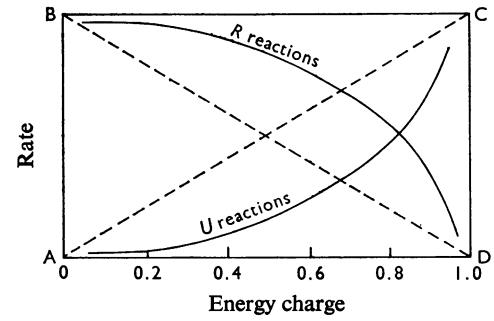

Fig. 5. *Relationship between the energy charge of the adenylate system and the rates of ATP-regenerating and ATP-utilizing systems*

Rates are expressed in arbitrary units; energy charge (Atkinson, 1968) is defined in the text. The solid lines depict typical relationships between energy charge and the rates of ATP-regenerating (*R*) and ATP-utilizing (*U*) systems. The area BCD encloses all possible *R* reactions, and the area ACD encloses all possible *U* reactions.

These results emphasize how important the maintenance of an adequate energy charge is in the cell, and from this point of view the sensitivity of the brain to anoxia may be readily understood. The brain is metabolically more active than most other tissues, but it does not have a correspondingly higher total adenine nucleotide content. It conducts a very active 'economy' with only a small amount of 'currency' in circulation. In a 'run' on the ATP the brain finds itself without sufficient reserves either of ATP or the 'convertible currencies' phosphocreatine and glycogen (in which it is also relatively poor) to maintain a high energy charge. Thus anoxia presents a particularly serious threat to the brain. Fig. 4 shows that anoxia decreased the energy charge of the whole rat brain to its lowest possible steady-state value in 1.6min; in the more metabolically active regions of the brain this lower limit could have been reached much earlier. In

this way the selective vulnerability of certain grey regions of the brain (e.g. the cerebral cortex and the Purkinje cells) may be explained.

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