Resting and Stimulated Respiration *in vitro* in the Central Nervous System

REGIONAL DISTRIBUTION AND RELATION TO CELL DENSITY

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(Received 1 May 1967)

1. The respiratory rates *in vitro* of ten structures of the rabbit brain were measured in the presence of a normal (5 mM) and an elevated (50 mM) concentration of K^+ ions. The results were expressed on a dry-weight basis and in terms of cell density. 2. On a dry-weight basis, with a normal concentration of K^+ , there was a steady decrease in respiratory rate on passing from the cerebral cortex through successively lower centres to the brain-stem, i.e. respiration was negatively correlated with phylogenetic age. 3. When the resting respiratory rates were expressed in terms of cell density there was no correlation with phylogenetic age. The neuron-containing structures of the cerebrum and brain-stem had identical respiratory rates with the exception of the cerebral cortex which had a higher rate. 4. The K^+ -stimulated respiratory rates/cell also showed a negative correlation with phylogenetic age; the higher centres responded with a greater percentage increase than the lower. 5. The results from the cerebellar cortex were anomalous. 6. There were variations of respiratory rate within the diencephalon. The thalamus had a relatively high respiratory rate, the anterior and ventral regions relatively low and the hypothalamus intermediate. 7. The oxygen consumption of white matter was not increased by a high external concentration of K^+ .

It is widely believed that in the course of phylogeny the newer brain structures have developed a more aerobic metabolism. The most direct evidence for this is provided by regional variations in the respiration *in vitro* of brain tissue taken from ox (Dixon & Meyer, 1936), dog (Himwich & Fazekas 1941; Elliott & Heller, 1957), rat (Himwich, Sykowski & Fazekas, 1941), cat and man (Elliott & Heller, 1957) and calf (Hertz & Clausen, 1963). The absolute values vary from species to species, but the relative values are in good agreement; respiration/unit weight of tissue is highest in cortical tissue and decreases steadily through successively lower (i.e. phylogenetically older) structures down to the spinal cord. A similar variation was found (Ridge, 1967) for the activity of cytochrome oxidase (EC 1.9.3.1) in the rabbit brain. However, if the enzymic activity was expressed in terms of cell density (as measured by DNA), all the neuron-rich structures of the central nervous system except the cerebellar cortex showed the same cytochrome oxidase activity regardless of phylogenetic age. It was suggested that values expressed in terms of cell density more accurately reflected the cytochrome oxidase activity of the

nerve-cell body, since values expressed in terms of tissue weight were modified by the inclusion of tissue elements having little or no enzymic activity.

The same considerations apply to the respiration of nervous tissue. There were, therefore, two reasons for re-examining the respiration of tissue from the central nervous system: (1) to see whether the relationship between respiration and phylogenetic age still held if the former was expressed in terms of cell density; (2) to see whether an even distribution of enzymic activity was peculiar to cytochrome oxidase or whether it was a more general feature of the respiratory enzymes of the central nervous system.

A high concentration of K^+ in the incubating medium has long been known to stimulate the respiration *in vitro* of slices of cerebral cortex, but I have been unable to find any report of a systematic study of its effect on tissue from other regions of the central nervous system. I have therefore included such a study in the present work.

METHODS

Female New Zealand White rabbits, each weighing 3.2 ± 0.5 kg., were used. They were killed by an intravenous air injection.

Respiratory rate was measured in a standard constant-volume Warburg apparatus at 37°. Readings were taken

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at 5 min. intervals for 70 min. The incubation medium was a Krebs-Ringer salt solution prepared as described by Cohen (1959), except that the 0.1 M-phosphate buffer was replaced by an equal volume of 0.32 M-tris buffer (pH 7.4 at 37°). The portion of iso-osmotic KH_2PO_4 (solution 4) was retained. This gave a final K^+ concentration of 5 mM. Preliminary experiments showed that the respiration was unaffected by the change from phosphate to tris buffer. The gas phase was O_2 and the substrate was 11 mM-glucose.

In the high- K^+ -concentration experiments (final concn. 50 mM), KCl dissolved in tris-buffered Krebs-Ringer solution (as above) was added from the side arm 30 min. after normal readings had started. After 10 min. for the addition and re-equilibration, subsequent readings were taken every 5 min. for a further 30 min.

The brain regions examined were the cerebral cortex, the caudate nucleus, the hippocampus, the diencephalon, the mesencephalon, the pons, the medulla oblongata, the cerebellar cortex, the central white matter of the cerebellum and the corpus callosum. With the exception of the diencephalon all the regions were dissected out freehand. The region of the cerebral cortex used was that lying dorsal to the diencephalon. The sample of cerebellar cortex was taken from the median vermis and the central white matter of the cerebellum was teased out from the lateral hemispheres of the cerebellum. Immediately after excision the tissues were transferred to ice-cold Krebs-Ringer solution (as above, except that the tris buffer was adjusted to give pH 7.4 at 0°). The tissues were cut into slices 0.33 mm. thick on the tissue slicer previously described (Ridge, 1964). No more tissue was used for any one experiment than could be transferred to the Warburg flasks within 20–25 min. of the death of the animal. The structures were removed from the ice-cold Krebs-Ringer solution in random order. The variable time spent in the ice-cold solution had no detectable effect on the respiratory rate.

As the diencephalon, the mesencephalon, the pons and the medulla are all anatomically more or less complex, an attempt was made to examine the distribution of oxygen consumption within these regions.

The general method of stereotactic dissection (Ridge, 1964) was applied to the diencephalon (Ridge, 1967). This yielded a rectangular block of tissue bounded by planes A3, P4, L3, R3, D3 and the ventral surface of the brain. The planes of section are described by the number of millimetres they lie anteriorly (A), posteriorly (P), dorsally (D), ventrally (V), to the left (L) or to the right (R) of previously defined (Ridge, 1964) frontal, horizontal or sagittal reference planes. In one series of experiments slices were cut from the tissue block parallel to the frontal reference plane at 0.33 mm. intervals. Each set of three adjacent slices, corresponding to 1 mm. depth of tissue, was placed in a different Warburg flask. In two further series, similar sets of slices were cut parallel to the sagittal and horizontal reference planes respectively.

The mesencephalon was defined as that region of the brain-stem bounded rostrally by a plane containing the posterior commissure and cutting the ventral surface of the brain just caudal to the mammillary bodies and caudally by a plane normal to the direction of the aqueduct and just caudal to the inferior colliculi. Four sets of six 0.33 mm. slices were cut horizontally and each set of slices was allocated to a different Warburg flask.

The pons, which is clearly delineated, was divided into

three sets of six 0.33 mm. slices cut in horizontal planes and the medulla into three sets of six 0.33 mm. slices cut in frontal planes. The slices composing each set were incubated together.

The respiratory rates on a DNA basis were derived from those on a dry-weight basis by using dry-weight DNA concentrations calculated from the fresh-weight DNA concentrations (Ridge, 1967) and the tissue water contents given in Table 1. The water content of the tissues was estimated by drying to constant weight at 105°.

RESULTS

Preliminary experiments showed that in all regions of the brain the unstimulated respiratory rate *in vitro* was maximal over the range 5.5–22 mm-glucose. Therefore 11 mm-glucose was used as the substrate in all the subsequent experiments. The principal results are presented in Table 1.

Unstimulated respiration. The unstimulated respiratory rate, expressed in terms of dry weight, decreased steadily from the cerebral cortex down to the midbrain and thereafter remained steady, i.e. it decreased in order of increasing phylogenetic age. The respiratory rate of the corpus callosum, a neuron-free structure, was significantly lower than that of any other brain region examined, including the central white matter of the cerebellum, which might have been expected to respire at about the same rate. That it did not is probably due partly to the difficulty of freeing it completely from the granular layer of the cerebellar cortex and partly to the neuronal aggregates embedded in it. The cerebellar cortex respired as rapidly as the cerebral cortex although it is a very much older structure.

In terms of cell density, the respiratory rate of the cerebral cortex was significantly higher than that of any other structure. Next in order, and significantly lower, was a group of six structures with identical oxygen consumptions: the caudate nucleus, the hippocampus, the diencephalon, the mesencephalon, the pons and the medulla oblongata. Significantly lower than this group was another consisting of the cerebellar cortex, the central white matter of the cerebellum and the corpus callosum. (In this context, 'significant' means that the ranges indicated in *italics* in Table 1 did not overlap.)

K⁺-stimulated respiration. In the presence of 50 mM- K^+ , the respiratory rates of three of the structures (the pons, the corpus callosum and the central white matter of the cerebellum) were unchanged and the remaining seven showed statistically significant increases ($P < 0.01$). The greatest percentage increases were in the cerebral and cerebellar cortices, with steadily decreasing percentage increments towards the brain-stem.

Within the diencephalon, there were wide spatial variations in respiratory rate. Fig. 1 shows: (i) that

Table 1. *Unstimulated and K⁺-stimulated respiration of different regions of the rabbit brain*

The dry weight values are expressed as the mean $\pm$ S.E.M. with the number of animals in parentheses. The respiratory rates on a DNA basis were calculated from those on a dry-weight basis, the fresh-weight DNA concentration and the water content of the tissue (column 2). The *italicized* figures indicate the extreme range of values calculated by allowing the dry-weight respiratory rates and the DNA concentrations to vary by ± 1 S.E.M. in opposite directions.

Brain region	Water content (%, w/w)	Oxygen consumption				K ⁺ -stimulated increment (%)
		Unstimulated		K ⁺ -Stimulated		
		(μ l. of O ₂ /mg. dry wt./min.)	(μ l. of O ₂ / μ g. of DNA/min.)	(μ l. of O ₂ /mg. dry wt./min.)	(μ l. of O ₂ / μ g. of DNA/min.)	
Cerebral cortex	79.9	0.219 $\pm$ 0.011 (8)	0.049 $\pm$ 0.005	0.404 $\pm$ 0.019 (8)	0.090 $\pm$ 0.009	+ 84
Caudate nucleus	80.2	0.190 $\pm$ 0.007 (8)	0.030 $\pm$ 0.003	0.320 $\pm$ 0.021 (8)	0.051 $\pm$ 0.007	+ 70
Hippocampus	77.7	0.160 $\pm$ 0.010 (8)	0.037 $\pm$ 0.005	0.242 $\pm$ 0.018 (8)	0.056 $\pm$ 0.007	+ 51
Diencephalon	76.6	0.134 $\pm$ 0.008 (10)*	0.029 $\pm$ 0.005	0.201 $\pm$ 0.010 (8)*	0.044 $\pm$ 0.005	+ 51
Mesencephalon	73.4	0.079 $\pm$ 0.006 (8)*	0.032 $\pm$ 0.005	0.111 $\pm$ 0.002 (8)*	0.045 $\pm$ 0.003	+ 41
Pons	70.6	0.075 $\pm$ 0.007 (8)*	0.031 $\pm$ 0.006	0.078 $\pm$ 0.008 (8)*	0.032 $\pm$ 0.007	+ 4
Medulla oblongata	72.7	0.078 $\pm$ 0.002 (8)*	0.031 $\pm$ 0.002	0.099 $\pm$ 0.006 (8)*	0.040 $\pm$ 0.003	+ 27
Cerebellar cortex	79.5	0.247 $\pm$ 0.016 (8)	0.013 $\pm$ 0.002	0.433 $\pm$ 0.020 (8)	0.023 $\pm$ 0.002	+ 75
Central white matter of cerebellum	70.6	0.089 $\pm$ 0.007 (8)	0.018 $\pm$ 0.003	0.078 $\pm$ 0.009 (8)	0.016 $\pm$ 0.003	- 12
Corpus callosum	73.0	0.051 $\pm$ 0.007 (8)	0.012 $\pm$ 0.002	0.046 $\pm$ 0.004 (8)	0.010 $\pm$ 0.002	- 10

* These values are the weighted mean for the whole structure calculated from the sets of slices (see the text).

there was no significant lateral variation (Fig. 1a); (ii) that rostrocaudally the respiratory rate increased steadily from A3 to AP0 and thereafter remained steady (Fig. 1b); and (iii) that dorsoventrally there was a maximum between about V3 and D1 or D2, with much lower respiratory rates ventrally but only slightly lower rates dorsally. Figs. 1(a), 1(b) and 1(c), considered together, indicate that there was a region of relatively high respiratory rate extending from AP0 to P4 rostrocaudally, from V3 to about D2 dorsoventrally and from L3 to R3 laterally. The anterior and ventral parts of the tissue block showed a relatively low respiratory rate. Since there was no evidence of a sharp discontinuity between the two extremes, it was assumed that the tissue between them respired at an intermediate rate. The distribution of respiratory rates is the reverse of that of cell density (Ridge, 1967), so that if the respiratory rates/cell were plotted as in Fig. 1 the same picture would emerge except that the vertical scale would be exaggerated.

Reference to the stereotactic atlas of the rabbit brain (Ridge, 1964, 1967) shows that the region of high respiratory rate corresponds to the thalamus. The low-respiratory-rate region includes such structures as the optic tract and the anterior commissure as well as one or two rather diffuse nuclei. The principle structure in the region of intermediate respiratory rate is the hypothalamus. The distribution of oxygen uptake closely resembles that of cytochrome oxidase activity (Ridge, 1967).

The stimulating effect of high K⁺ concentration was reasonably uniform within the diencephalon (Fig. 1c). The relatively small effect in region V5-V4 is perhaps associated with the presence of the optic tract in this region (cf. the lack of stimulation by 50 mM-K⁺ on the corpus callosum; Table 1).

No spatial variation of oxygen consumption was found in the mesencephalon, the pons or the medulla. Whether any such variation would have been found had the slices been cut in planes other than those in which they were cut is an open question, but it seems unlikely, since the planes chosen were those in which, on anatomical grounds, one would expect any respiratory differences to have been revealed.

DISCUSSION

On the basis of the results in Table 1, the structures investigated fall naturally, for the purposes of discussion, into three groups: (a) the neuron-containing structures of the cerebrum and brain stem, (b) the white matter and (c) the cerebellar cortex.

Within group (a), there was an obvious negative correlation between phylogenetic age and the oxygen consumption/unit weight of tissue, thus extending to the rabbit a similar relationship found in other mammalian species. However, when the respiratory rates/cell within this group were considered, the correlation disappeared. With the

Bioch. 1967, 105

Fig. 1. Oxygen consumption within the rabbit diencephalon. The diencephalon was examined in 1 mm. sections parallel to each of the stereotactic reference planes: (a) sagittal, (b) frontal and (c) horizontal. ○, 5mM-K⁺; ●, 50mM-K⁺. Each point was derived from six to ten animals. The vertical bars are 2 S.E.M. in length. The abscissae are stereotactic co-ordinates (Ridge, 1964).

exception of the cerebral cortex, which had a significantly higher rate, all the structures had the same respiratory rate. On stimulation with excess of K⁺ the correlation reappeared because the higher structures showed a greater proportional increase than the lower. The reason for this is not clear. Qualitative differences between the regions in respiratory enzymes is not to be expected, and it is generally accepted that they are present in the same relative proportions, a conclusion supported by the similarity in the resting respiratory rates/cell. Why then should the regions respond differently to a common stimulus? One explanation might be that 50mM-K⁺ is not an equivalent stimulus in each case. The neurons of the caudate nucleus may be more readily depolarized than, say, those of the medulla. Or perhaps one or more of the many endergonic reactions dependent on glycolysis, e.g. acetylcholine synthesis, is stimulated to different extents in different structures.

The results may also be taken to indicate that if the neurons of the various structures are in an environment in which they are continuously being stimulated, as *in vivo*, their oxygen requirements are in line with their stimulated oxygen consumption *in vitro*. The differential effect of K⁺ stimulation might well be related therefore to the well-known differential effect of oxygen deprivation in which the higher structures suffer greater damage than the lower.

The respiratory rate of white matter was predictably low and was unaffected by the K⁺ concentration of the incubating medium. This is perhaps not surprising if the effect of high K⁺ concentration is considered to be mediated, at least initially, by depolarization of the cell membrane, although subcortical white matter responds to electrical stimulation with an increased oxygen consumption (McIlwain, 1966).

The cerebellar cortex is considered separately

because it could not readily be included in either of the other groups. Phylogenetically it is an ancient neuron-containing structure yet its respiratory rate/unit weight was as high as that of the cerebral cortex and its respiratory rate/cell as low as the corpus callosum. On K^+ stimulation, its respiratory rate increased by 75%, a value intermediate between that of the cerebral cortex and the caudate nucleus. The cerebellar neurons, which are principally of two types, small very densely packed granule cells in a thick layer and large, geometrically regular Purkinje cells in a monolayer, differ from other neurons in other respects also. Thus the Purkinje cells are extremely sensitive to manipulation (they will not survive penetration by the $1.0\ \mu$ top of a micro-electrode for more than about 1 min.), to anoxia, to anaesthetics (particularly barbiturates) and to a wide range of other substances. The electroencephalograph trace for the cerebellum, which is believed to originate in the Purkinje cells, shows very rapid changes of potential (about 250/sec.), compared with the α -waves from the cerebral cortex, and is unaffected by arousal. The neurons of the granular layer, which far outnumber the Purkinje cells and therefore largely determine the biochemical profile of the tissue, are also somewhat atypical. They are smaller and more densely packed than other neurons and differ in at least one important biochemical feature: they have a much lower cytochrome oxidase activity (Ridge, 1967). It would appear therefore that the high respiratory rate/unit weight of the cerebellar cortex was related to its high cell density (6.16×10^5 cells/mg. fresh wt.) (Ridge, 1967), and not to a more highly evolved biochemical apparatus.

It appears that the relationship between K^+ -stimulated respiration and the effects of oxygen deprivation applies also to the cerebellar cortex, since this region is one of the most susceptible parts of the central nervous system to oxygen deficiency and it responds strongly to K^+ stimulation. But there is a difficulty: visible anoxic damage to the cerebellar cortex is restricted to the Purkinje cells (Kabat & Dennis, 1939), the granule cells appearing histologically normal. It is inconceivable that more than a small proportion of the total oxygen usage of the cerebellar cortex can be attributed to the Purkinje cells, since they are relatively so few in number. Most of the K^+ -stimulated respiration must therefore arise from the granule cells that are comparatively resistant to oxygen lack.

REFERENCES

- Cohen, P. P. (1959). In *Manometric Techniques*, p. 149. Ed. by Umbreit, W. W., Burris, R. H. & Stauffer, J. F. Minneapolis: Burgess Publishing Co.
- Dixon, T. F. & Meyer, A. (1936). *Biochem. J.* **30**, 1577.
- Elliott, K. A. C. & Heller, I. H. (1957). In *Metabolism of the Nervous System*, p. 286. Ed. by Richter, D. London: Pergamon Press Ltd.
- Hertz, L. & Clausen, T. (1963). *Biochem. J.* **89**, 526.
- Himwich, H. E. & Fazekas, J. F. (1941). *Amer. J. Physiol.* **132**, 454.
- Himwich, H. E., Sykowski, P. & Fazekas, J. F. (1941). *Amer. J. Physiol.* **132**, 293.
- Kabat, H. & Dennis, C. (1939). *Amer. J. Physiol.* **126**, 549.
- McIlwain, H. (1966). In *Biochemistry of the Central Nervous System*, pp. 54, 55. London: J. and A. Churchill Ltd.
- Ridge, J. W. (1964). *J. Neurochem.* **11**, 765.
- Ridge, J. W. (1967). *Biochem. J.* **102**, 612.