Biochem. J. (1963) **89**, 526

Effects of Potassium and Sodium on Respiration: their Specificity to Slices from Certain Brain Regions

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(*Received* 4 *March* 1963)

Slices of brain tissue show an increased rate of oxygen uptake during incubation in media that contain high concentrations of potassium. Dickens & Greville (1935), using slices from brain, liver, kidney, testis or tumour tissue, found this effect only in brain. Some investigators (e.g. Hegnauer, Fenn & Cobb, 1934) have observed a similar stimulation of respiration when muscle is exposed to high potassium concentrations, although others (e.g. Zierler, 1956) have not observed this effect.

Hertz & Schou (1962) have found that the increased oxygen uptake represents only one aspect of the effect of potassium on the respiration of brain slices. This initial stimulation of oxygen uptake in the potassium-rich media is followed by a very rapid decline in the rate of oxygen uptake, which falls below that of slices respiring in media with low potassium concentrations. A similar rapid fall of oxygen uptake was observed in media containing 'unphysiologically' low concentrations of sodium.

One aim of the present study was to find out whether the rapid fall of the respiration in the 'unbalanced' media is specific to brain slices or whether other tissues react in a similar way. The stimulating effect of high potassium concentrations on the oxygen uptake of muscle was confirmed; however, the subsequent courses of the initially stimulated respiration in brain slices and in muscle were found to differ.

A second aim was to study whether the changes in rate of oxygen uptake observed in potassium-rich or sodium-free media are found with slices obtained from all parts of the central nervous system or whether they are specific to certain regions. Such a specificity might be expected since no increase in rate of oxygen uptake is found when peripheral mammalian nerve is exposed to high potassium concentrations.

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METHODS

Rats of the Wistar strain weighing 100–200 g. were decapitated while under light ether anaesthesia. The kidneys, the brain, the spinal cord, the two halves of the diaphragm or the sciatic nerves were removed immediately. The two last-named preparations were introduced directly into Warburg vessels; the spinal cord was isolated and cut into pieces as described by Rafaelsen (1958) before the transfer to the incubation vessels. Kidney and brain slices were prepared without moistening by means of a Stadie–Riggs microtome designed to cut slices about 0·5 mm. thick (Stadie & Riggs, 1944). From each kidney several slices were cut from a surface exposed by a section from the pelvis renis to the cortex. Accordingly, the proportions between cortex and medulla varied from one slice to another, but this appeared to affect only the absolute level of the rate of the oxygen uptake and not the reactions to variations in the sodium and potassium concentrations. From each brain hemisphere, however, only one slice was cut. This slice was the first one obtained when cutting from the outer convexity of the lateral surface.

The fresh weights of the tissues were found by determining the differences between the weights of the vessels, containing 3·0 ml. of medium, before and after introduction of the preparations. Usually slicing and weighing took place in a cold room (2–4°), but in a few experiments brain slices or hemidiaphragms were transferred directly to incubation vessels containing warm oxygenated media. At about 15–30 min. after the decapitation of the rats the vessels were placed in a water bath at 37°. They were oxygenated for 5 min. and thereafter equilibrated for a further 5–10 min.

Owing to the small size of the rat brain it was not possible to study differences between various parts of the central nervous system in detail with this animal. More detailed studies were therefore performed with slices from the calf brain. Brains and cervical spinal cords from calves were obtained at the common cattle market. Portions of the brains were damaged, since the animals were shot through the head. This damage was consistently localized to areas far removed from those selected for study.

The calf was quickly beheaded, the skull cleft transversely and the brain removed together with a few centimetres of adhering spinal cord. Tissue blocks were cut from the selected areas and were placed in ice-cold media about 5 min. after the calf was killed. The slicing and the incubation were similar to that employed with rat tissue, except that the incubations could not be started until about 1 hr. after the death of the calf and that often several slices were

incubated in each Warburg vessel to obtain an oxygen uptake of sufficient magnitude.

Slices were cut from the following parts of the calf central nervous system: (1) Cervical spinal cord. The spinal cord was cut frontally. In some experiments the slices were transferred directly to the incubation vessels; in other experiments predominantly grey matter was isolated in the form of two strips from each of the central slices. About six such strips were generally incubated in one Warburg vessel. (2) Medulla oblongata. Slices were cut transversely or frontally through the entire medulla oblongata. No attempt at further isolation was made. (3) Pons. The brain stem was sectioned transversely 1 cm. below the rostral border of the pons, which was easily recognized macroscopically. Slices were cut from this surface in a caudal direction. (4) Upper border of pons. A transverse section was made at the rostral border of the pons and slices were cut from here in a caudal direction. (5) Mesencephalon. Slices were cut transversely through the entire mesencephalon. Slices from the colliculi, which were easily recognized macroscopically, were studied separately. (6) Colliculi. Slices were cut from the dorsal surface in a ventral direction. (7) Caudate nucleus. A large structure of grey matter was consistently found adjacent to the third ventricle, and histological examination verified that it was the caudate nucleus. Slices were cut from the free ventricular surface of this structure in a lateral direction; the first slice was not used owing to the presence of a layer of ependyma cells. (8) Cerebral white matter. Blocks of suitable size could easily be obtained from the centrum semiovale. Slices were cut from these blocks in random directions. (9) Cerebral cortex. The arachnoideal vessels and membranes were removed and slices were cut from the pia-covered surface of preparations from (*a*) frontal pole, (*b*) anterior central gyrus, (*c*) posterior central gyrus, and (*d*) occipital pole. (10) Cerebellum. Slices of cerebellar cortex were cut from the surface of the cerebellar hemispheres after removal of the arachnoideal vessels and membranes.

The rate of oxygen uptake by the slices was determined with conventional Warburg constant-volume respirometers. The manometers were read every 10 min. The rates of oxygen uptake were expressed as μmoles/g. fresh wt./hr., usually as the average of three 10 min. periods. Frequently the incubations lasted several hours and the manometers had to be readjusted to their starting points. The first 10 min. period after each adjustment was disregarded.

Media were prepared from solutions of purest-grade chemicals. Usually the chlorides were used, but magnesium was used as its sulphate. Owing to the hygroscopicity of lithium chloride and of calcium chloride, the exact concentration of stock solutions of these salts was determined by a chloride titration. The sodium concentration in the media was usually 120–125 mM. In the sodium-free medium sodium was replaced by an equiosmolar concentration of sucrose. The media were prepared either without potassium or with a potassium concentration of 5 mM; the 'potassium-free' medium rapidly acquired a potassium concentration of about 2 mM owing to leakage from the cells. The calcium concentration was 1·5 mM and the magnesium concentration 1·0 mM. The media generally contained glucose (6 mM) but one set of experiments was performed with a glucose concentration of 25 mM to be sure that the fall in respiration during prolonged incubation was not due to any lack of glucose. The media were buffered with 20 mM-tris [tris-(hydroxymethyl)aminomethane plus tris(hydroxymethyl)-aminomethane hydrochloride] to pH 7·4. The pH was checked before and after the incubation.

RESULTS

Respiration of tissues from the rat. The rate of oxygen uptake of rat-brain-cortex slices during prolonged incubation in media of different cation compositions is shown in Fig. 1. The respiration is fairly well maintained in a medium containing sodium (120 mM) and potassium (5 mM), since after 5 hr. of incubation in this medium the oxygen uptake has only fallen to about 60 % of its initial value. The addition of further potassium chloride (equivalent to a final concentration of 48 mM) to the same system during the incubation produces an initially high rate of oxygen uptake. But the 'stimulated' respiration declines rapidly, and after 5 hr. the 'stimulated' rate of oxygen uptake has fallen significantly to about two-thirds of the 'unstimulated' respiration. Replacement of all the sodium chloride in the medium with sucrose leads to an even more rapid decrease of the rate of oxygen uptake until, after the first 2 hr. of incubation in the sodium-free medium, the respiration becomes relatively stable at about 10 % of the initial value.

These effects are unique to brain tissue. Somewhat similar phenomena are found in experiments with muscle but not with kidney slices. Fig. 2 shows the respiration of kidney and brain slices and of hemidiaphragms before and after the addition of further potassium chloride (equivalent to a final concentration of 48 mM) to tissue respiring in the medium containing sodium (120 mM) and potassium (5 mM). With kidney the raised potassium concentration causes a slight decrease in the oxygen uptake. This decrease is already evident during the

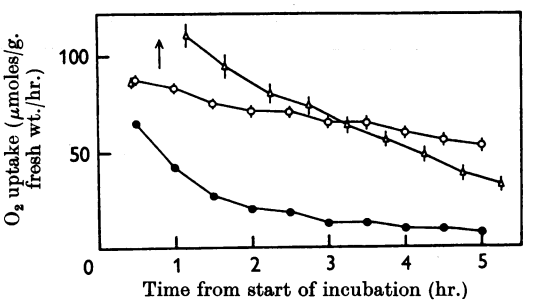

Fig. 1. Rates of oxygen uptake by rat-brain slices during incubation in media containing potassium chloride (5 mM) and either sodium chloride (120 mM) (○, △) or sucrose (250 mM) (●). The concentration of glucose is in this experiment 25 mM. In one experiment (△) potassium chloride (equivalent to a final concn. of 48 mM) was added during the incubation (arrow). S.E.M. are indicated by vertical bars if they extend beyond the symbols. Results are the means of values obtained with seven to eleven slices.

first period recorded after the addition of potassium. The 10 min. period during which the potassium was added was disregarded, since the addition to several vessels takes several minutes. Accordingly the tissue was only exposed to a high potassium concentration during the latter part of this 10 min. period. The respiration is well maintained even after the addition of potassium. The slight decline in respiratory rate which occurs is exponential and is similar to the corresponding decline in a 'balanced' medium (cf. Fig. 4). With brain slices and hemidiaphragms a marked initial stimulation and a subsequent increased rate of respiratory decline is observed after the addition of potassium.

The 'stimulated' respiration of hemidiaphragms declines rapidly, so that within 1 hr. after the addition of potassium the rate of oxygen uptake is the same as it would have been if no potassium had been added. The gradual decline to the level of the 'unstimulated' preparation is not exponential; the oxygen uptake in the first 10 min. after the addition of potassium is slightly underestimated because the time required to add the potassium is included in this period. Once the oxygen uptake of the muscle has reached a level similar to that before the addition of potassium, the respiration is, however, almost as well maintained as in 'balanced' media and shows only a slight exponential fall with time. These two phases of the decline in oxygen consumption after the addition of potassium were consistent findings in our experiments with muscle. Though the magnitude of the stimulatory effect varied in different preparations the qualitative effect shown in Fig. 2 is significant ($P < 0{\cdot}05$).

The rate of oxygen uptake of brain-cortex slices is increased about 60 % by the addition of potassium and subsequently declines in an exponential manner, which remains unchanged from the first 10 min. period to the end of the incubation. The initially 'stimulated' rate of respiration eventually falls well below that of slices incubated in media with low potassium concentrations (cf. Fig. 1).

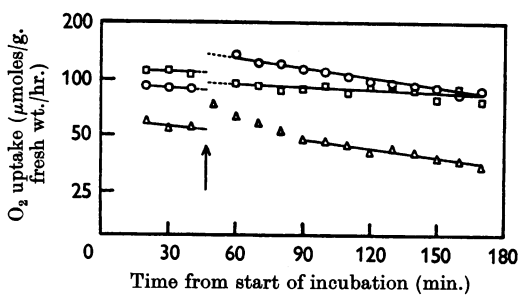

Fig. 2. Rates of oxygen uptake by kidney slices (□), brain slices (○) and hemidiaphragms (△), initially respiring in a medium containing potassium chloride (5 mM) and sodium chloride (120 mM). Potassium chloride (equivalent to a final concn. of 48 mM) was added during the incubation (arrow). Results are the means of values obtained with four to ten slices.

A similar initial stimulation and subsequent rapid decline of the rate of oxygen consumption was observed after the addition of further potassium chloride (equivalent to a final concentration of 48 mM) to slices from the cerebellum of the rat. On the other hand, the addition of potassium to sciatic nerve or to pieces of spinal cord lowered rather than raised the rate of oxygen uptake. Pieces of spinal cord were also cut into halves in a longitudinal direction to counteract anoxia of the core and to secure its exposure to potassium. The rate of respiration was more than doubled, but even under these conditions there was no further stimulation when further potassium chloride was added to give a final concentration of 50 mM.

Respiration of regions of calf central nervous system. The rate of oxygen uptake in different regions of the central nervous system was studied in more detail with slices from calf brain. The caudate nucleus responded to the addition of further potassium with a rate of oxygen uptake which is almost doubled when the potassium concentration in the medium is about 50 mM. Potassium concentrations between 20 and 50 mM cause a submaximum response; concentrations above 50 mM cause a maximum or slightly submaximum stimulation that is followed by a very rapid decline.

That the metabolic response to potassium reaches its maximum at a potassium concentration of 50 mM agrees with previous findings on rat-brain cortex. The sensitivity of other parts of the calf brain to potassium was therefore also studied by the addition of further potassium chloride (equivalent to a final concentration of 48 mM). Rates of respiration in two identical groups of slices from each region were compared. To one group further potassium chloride (equivalent to a final concentration of 48 mM) was added after 30 min. of incubation, whereas no addition was made to the slices in the other group. The procedure followed for the calculation of the degree of stimulation is illustrated in Fig. 3 (□, ○), which shows the respiration in two groups of slices from the caudate nucleus. In experiments with individual slices from the cerebral cortex, caudate nucleus and colliculi, the rate of oxygen uptake rose significantly after the addition of further potassium; slices from the rest of the mesencephalon and from the upper border of the pons also appeared 'stimulated' but this was not statistically significant. The decline in respiration with time was usually somewhat more rapid than the corresponding decline in a 'balanced' medium, which could be followed in the control groups of

slices. The procedure illustrated in Fig. 3 (□, ○) was therefore followed for the calculation of the degree of stimulation at the time when the further potassium chloride was added.

The collected results of experiments with slices from different regions in the calf brain are shown in Table 1. The rate of respiration of slices from cerebral and cerebellar cortex is increased about 70% by the addition of further potassium. The caudate nucleus shows an even larger increase in the rate of oxygen uptake, whereas slices from the mesencephalon and the upper border of the pons are only slightly 'stimulated'. Slices from the rest of the pons, the medulla oblongata and the spinal cord show no stimulation of the rate of respiration when further potassium chloride (equivalent to a final concentration of 48 mM) is added.

The potassium-induced stimulation is only found in the regions that contain grey matter. The rate of respiration of white matter from the centrum semiovale and from the spinal cord does not show any stimulation on the addition of further potassium chloride (equivalent to a final concentration of 48 mM). However, the presence of nerve cells in the slices is not always accompanied by respiratory stimulation when potassium is added, since Table 1 shows a decreasing response from the rostral to the caudal parts of the central nervous system; and slices of grey matter from the spinal cord are not at all stimulated on the addition of potassium.

Also caesium, rubidium, and lithium are, in sufficiently high concentrations, able to cause an increased rate of respiration in brain-cortex slices (e.g. Hertz & Schou, 1962) and in muscle (Muller & Simon, 1960). The concentration of lithium required for a maximum stimulation of the rate of respiration in rat-brain-cortex slices is about 100 mM. This 'stimulated' rate of oxygen uptake is of the same magnitude as that after the addition of further potassium chloride (equivalent to a final concentration of 48 mM). A few experiments in which lithium chloride (to give a final concentration of 96 mM) was added to slices from the calf brain showed, however, that the increase in the rate of oxygen uptake is only about one-quarter of that after the addition of potassium chloride. An increase in the lithium concentration above 100 mM does not increase the respiration any further. No differences between slices from the cortex and those from the caudate nucleus were found in the effects of lithium.

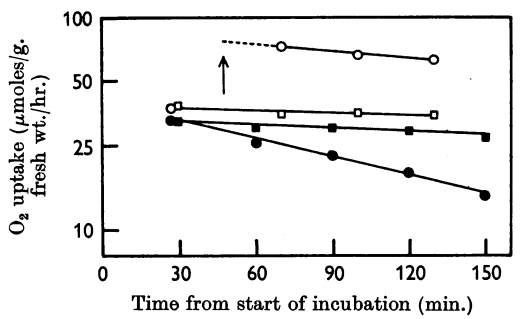

Fig. 3. Rates of oxygen uptake by slices from calf caudate nucleus during incubation in sodium-containing (□, ○) or sodium-free (■, ●) media. The media contained: sucrose (250 mM) (■), sucrose (250 mM) together with potassium chloride (5 mM) (●), or sodium chloride (125 mM) (□, ○). In an experiment (○) potassium chloride (equivalent to a final concn. of 48 mM) was added during the incubation (arrow). The broken line indicates extrapolated rates of oxygen uptake, and the stimulation expressed in Table 1 is the difference between the lines ○–○ and □–□ at the time of the addition of potassium. Results are the means of values obtained with three to seven slices.

Correlation between the effects of potassium and sodium. The close resemblance between slices from the cortex and from the caudate nucleus when they are exposed to high concentrations of stimulating ions is not found when they are exposed to lack of sodium. Fig. 1 shows that the rate of respiration of rat-brain-cortex slices decreases rapidly when all of the sodium chloride in the medium is replaced with sucrose. In the experiment shown in Fig. 1 the slices were respiring in a medium containing potassium (5 mM), but a similar fall in respiratory rate occurs in a medium from which potassium has been omitted. The oxygen uptake of calf-brain-cortex slices is likewise halved after about 1 hr. of incubation in a sodium-free medium, whereas the respiration of slices from the caudate nucleus are able to maintain their rate of oxygen consumption under these conditions. This is, however, only so in media that are also potassium-free [Fig. 3 (■, ●)]. The rate of respiration falls rapidly if the medium contains potassium at a concentration of 5 mM.

Slices of pure white matter and of grey matter from the spinal cord, on the other hand, maintain their respiration rate well in sodium-free media whether potassium is present or not. The respiration of sciatic nerve is similarly well maintained under these conditions. Accordingly, the sensitivity to lack of sodium seems to be characteristic of those nervous structures that show an increased respiration in media containing high concentrations of potassium.

This correlation between the stimulation by potassium and the requirement for sodium is also found when different tissues are compared. Kidney slices do not show any increased rate of respiration in media with high concentrations of potassium, and their oxygen uptake is equally well maintained in sodium-free and in sodium-containing media (Fig. 4). The rate of oxygen consumption in hemidiaphragms, on the other hand, is increased in media containing high concentrations of potassium, and it falls rapidly when all the sodium chloride in

Table 1. *Rates of oxygen uptake by slices from different regions in the calf central nervous system*

From each region two similar groups of slices were incubated in identical media (with a potassium concentration of about 2·0 mM). Details are given in the text. After 30 min. of incubation potassium chloride was added to one group (the 'stimulated' group) to give a final concentration of 50 mM, whereas no addition was made to the other group. The respiratory rates at the time of the addition were obtained by extrapolation and the degree of stimulation was calculated as illustrated in Fig. 3. Differences in respiration between the two groups before the addition of potassium chloride (i.e. rate of oxygen uptake in 'stimulated' group minus rate of oxygen uptake in 'unstimulated' group) were corrected by a subtraction of the difference between the logarithms of these values from the logarithm of the extrapolated stimulated respiratory rate (i.e. a parallel displacement of the difference was made on the semilogarithmic graph). The numbers in parentheses refer to numbers of tissue samples. Often more than one tissue sample from the same area were studied in each calf, but in no case were the results obtained with less than two animals. A total of twenty-one calves was studied. Statistical significances were not calculated on the basis of the extrapolated corrected values but on the directly measured rates of oxygen uptake in the same group before and after the addition of potassium and are indicated in the text.

Tissue	O_2 uptake (μmoles/g. fresh wt./hr.) No KCl added	With KCl (48 mM) added	Stimulation (%)
Spinal cord	7·34 (2)	6·90 (6)	−6·0
Spinal cord (predominantly grey matter)	22·97 (2)	21·19 (5)	−7·7
Medulla oblongata	11·19 (5)	10·15 (11)	−9·3
Pons	14·72 (7)	14·80 (8)	0·5
Pons (upper border)	12·62 (4)	15·32 (5)	21·4
Mesencephalon	23·36 (8)	31·23 (14)	33·7
Colliculi	32·22 (4)	44·22 (5)	33·1
Caudate nucleus	36·90 (3)	77·52 (4)	110·1
Cerebral white matter	7·78 (9)	7·67 (9)	−1·4
Frontal-lobe cortex	41·68 (2)	75·21 (3)	80·4
Occipital-lobe cortex	39·69 (3)	67·25 (4)	69·4
Motor cortex	43·78 (2)	70·84 (5)	61·8
Sensory cortex	44·57 (2)	73·30 (5)	64·5
Cerebellar cortex	44·28 (2)	79·98 (2)	80·6

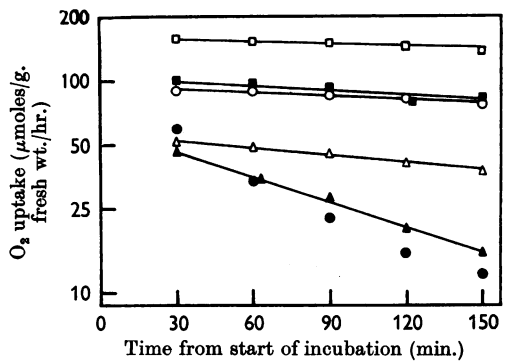

Fig. 4. Rates of oxygen uptake by kidney slices (□, ■), brain slices (○, ●) and hemidiaphragms (△, ▲) during incubation in media containing potassium chloride (5 mM) and either sodium chloride (120 mM) (□, ○, △) or sucrose (250 mM) (■, ●, ▲). Results are the means of values obtained with four to nine slices.

the medium is replaced with sucrose. This decline in the rate of the oxygen uptake is almost as rapid as that found with brain slices under similar conditions.

However, one qualitative difference in the respiratory effects induced by alteration of the sodium concentration in the medium is found between brain and muscle. These two tissues react in opposite ways to hyperosmotic sodium concentrations. The rate of oxygen uptake in hemidiaphragms is more than 50 % higher in a solution with a sodium concentration of 250 mM than in one with a sodium concentration of 120 mM; and it is equally well maintained, the fall in respiratory rate being only about 25 % during 2 hr. of incubation. The respiration of brain slices is, in contrast, slightly depressed in all media containing sodium concentrations ranging from 150 to 250 mM.

DISCUSSION

Kidney slices, hemidiaphragms and brain slices are all able to maintain their respiratory activity during several hours of incubation *in vitro*. When each of these three preparations is incubated in a medium with a cationic composition resembling that of extracellular fluid the rate of oxygen uptake declines only slowly. Deviations in the sodium and potassium concentrations in the medium from this 'balanced' state affect the respiratory rate of kidney, muscle and brain tissue in different ways.

In kidney slices the respiration is relatively insensitive to environmental changes in the concentrations of the two alkali-metal ions. The only effect we found when the sodium or potassium concentrations in the medium were changed was a decreased level of the oxygen uptake in the sodium-free medium. Since we were interested in the course of the respiration rather than in its absolute size no efforts were made to obtain pure cortical or medullary slices. Therefore the rate of oxygen uptake in different slices was variable and no quantitative evaluation of this sodium effect on respiration can be made. However, a qualitative agreement with previous findings on the whole isolated kidney (Thaysen, Lassen & Munck, 1961) and on kidney-cortex slices (Lassen & Thaysen, 1961) is apparent. The fact that the respiration remains as stable in the medium without sodium as in that with sodium (120 mM) is also in agreement with results by these investigators, since J. H. Thaysen & U. V. Lassen (personal communication) found a well-maintained oxygen consumption with kidney-cortex slices respiring in a medium in which all of the sodium had been replaced with lactose.

An unaltered or slightly decreased respiration of kidney slices in media with high concentrations of potassium has been described by several investigators (e.g. Dickens & Greville, 1935; Taggart, Silverman & Trayner, 1953; Mudge, 1951; Kleinzeller, 1961). Similar effects have been found in experiments with liver (Elliott & Bilodeau, 1962), testis and tumour tissue (Dickens & Greville, 1935). In contrast, Aebi (1953) has reported that high concentrations of potassium in the medium cause an increased respiration of liver and kidney slices; however, his experimental conditions differ from those usually employed, since he used a four-substrate mixture of pyruvate, fumarate, glutamate and glucose.

We have found that the respiration of peripheral nerve is altered in a manner similar to that of kidney slices when exposed to high external concentrations of potassium. Similar results have been reported by Chang, Shaffer & Gerard (1935) and by Pietra (1961), but Shanes & Hopkins (1948) found a potassium-induced increase of respiration of desheathed crab nerve. Thus interspecies variations may exist.

Studies on the oxygen consumption in muscle have not yielded unequivocal information. Several investigators have described a potassium-induced stimulation of respiration (Fenn, 1929; Hegnauer *et al.* 1934; Keynes & Maisel, 1954; Muller & Simon, 1960), but others have not found any correlation between the external potassium concentration and the rate of oxygen uptake. For example, Lee, Yu, Lee & Burstein (1961) found the rate of respiration in cat papillary muscle to be lower in a medium with a potassium concentration of 24 mM than in a medium with a potassium concentration of 6 mM, and Zierler (1956), using rat hemidiaphragms, found the same oxygen uptake (about 48 μmoles/g. fresh wt./hr.) in media with high and low potassium concentrations. This respiratory rate corresponds reasonably well with our 'unstimulated' oxygen uptake of 55 μmoles/g. fresh wt./hr. The transient stimulation of the rate of oxygen uptake which we found after the addition of further potassium chloride (equivalent to a final concentration of 48 mM) is not necessarily contradictory to Zierler's (1956) results, since the hemidiaphragms in her experiments were exposed to a high potassium concentration from the start of the incubation, whereas we added potassium during the incubation. That this difference in technique may be of importance was shown by Mullaney (1961), who found no increased oxygen uptake in frog muscle that was exposed to high concentrations of potassium from the start of the incubation but did find a distinct stimulation if the potassium were added during the incubation.

The 'stimulated' rate of oxygen uptake in the hemidiaphragms declines quickly to a level that is similar to the respiratory rate in a 'balanced' medium. Once this level has been reached the oxygen uptake is well maintained. A similar course of respiration is obvious in Fenn's (1929) report on frog sartorius muscle, and Muller & Simon (1960), also using frog sartorius muscle, found a respiratory stimulation that was only slightly decreased after 2 hr. of incubation in a medium with a potassium concentration of 22 mM. On the whole frog muscles seem more capable than mammalian muscles of maintaining respiration during incubation in 'unbalanced' media. Their rate of oxygen consumption is thus maintained for several hours at a high level in a sodium-free medium (Muller, 1962). This is in obvious contrast with our findings with rat hemidiaphragms. But it seems to be common to frog and mammalian muscle that the respiratory functions are at least not inhibited in media with fairly high concentrations of potassium. Further evidence that muscle cells may maintain their physiological properties during prolonged incubation in media with high concentrations of potassium is that they are able to maintain a normal gradient between the internal and external potassium concentrations under these conditions (Adrian, 1960).

With brain slices, in contrast, the high potassium concentrations interfere with the functions of the cells. A normal potassium gradient cannot be maintained (Pappius & Elliott, 1956) and, the rate of oxygen uptake falls rapidly from its initially 'stimulated' level to values below the 'unstimulated' respiration.

We have found that only slices containing grey

matter from the cerebrum, cerebellum or the brain stem show a potassium-induced respiratory stimulation. This is at variance with the results of Bollard & McIlwain (1957) and of Kurokawa (1960), who demonstrated that white matter from the guinea pig almost doubled its respiratory rate when exposed to increased concentrations of potassium. We have confirmed these results with guinea-pig white matter. This difference between white matter from the guinea pig and that from the calf could be due to interspecies variations, or it may be difficult to obtain a preparation containing only pure white matter from the rather small guinea-pig brain. The fact that this preparation shows a very high rate of oxygen uptake (half of that shown by grey matter; Bollard & McIlwain, 1957) may indicate a contamination with nerve-cell bodies. The respiratory rate we found in calf brain is about one-fifth of that shown by grey matter. This value agrees well with indirect measurements on the human brain (Allen, 1957).

Slices from the brain regions that are stimulated by high concentrations of potassium require a minimum concentration of sodium in the medium to maintain their respiration during prolonged incubation. The only exception is that slices from the caudate nucleus seem to maintain their rate of oxygen uptake well in a sodium-free medium; but this is probably due to traces of sodium in the medium, since the respiration declines rapidly if the sodium-free medium contains potassium at a concentration of 5 mM; a competition between sodium and potassium at the site where sodium exerts its effect on the maintenance of the respiration has been suggested by Hertz & Schou (1962).

A rapid decline in the rate of oxygen uptake is similarly found in media that contain ouabain in low concentrations (Wollenberger, 1947; L. Hertz, unpublished work); it is also seen when brain slices are incubated in 'potassium-free' media from which the potassium (1–2 mM) generally present due to leakage from the cells has been removed (Dickens & Greville, 1935; L. Hertz, unpublished work; Elliott & Bilodeau, 1962). This concentration (1–2 mM) of potassium is sufficient to maintain a normal respiration and a normal respiratory increase if high concentrations of potassium are added later on. Low external sodium concentrations, excessive potassium lack (Harris & Maizels, 1952) and ouabain (Schatzmann, 1953) all inhibit the active transport of sodium and potassium across the cell membranes. This transport seems therefore to be of vital importance for the respiration of brain tissue (cf. Whittam, 1961, 1962*a*).

The distribution of the potassium-sensitive regions within the central nervous system does not allow any conclusions as to which types of cells are stimulated. These may either be neurons or glia cells. In favour of the hypothesis that it is the glia cells that show the potassium-induced stimulation of the respiration is the elegant demonstration by Cummins & Hydén (1962) that a highly active ATP-splitting enzyme is found on the outside of the cell membranes of single isolated glia cells from the lateral vestibular nucleus.

Enzymes that split ATP and are stimulated by sodium and potassium at two different sites were first found in peripheral crab nerve (Skou, 1957), and have since been demonstrated in brain (e.g. Hess & Pope, 1957; McIlwain, 1962; Skou, 1962; Aldridge, 1962; Hess, 1962), muscle (Skou, 1962) and kidney (Whittam & Wheeler, 1961). They have also been thoroughly studied in erythrocytes (Post, Merritt, Kinsolving & Albright, 1960), where it has been shown that the potassium-sensitive site is located at the outside and the sodium-sensitive site at the inside of the membrane (Whittam, 1962*b*; Glynn, 1962). Much evidence has accumulated that these enzymes are involved in the active transport of sodium and potassium across cell membranes; and it has been suggested that the hydrolysis of ATP in kidney slices is closely linked to both respiration and active transport of sodium (Whittam, 1961). The low rate of oxygen uptake in kidney slices during incubation in sodium-free media is consistent with this hypothesis; and the respiratory rate in slices from the renal medulla increases with the sodium concentration in the medium up to about 500 mM (Ullrich & Pehling, 1958).

Incubation of muscle in media containing hyperosmotic concentrations of sodium causes similarly a stimulation of the respiration as shown by Muller (1962) and confirmed in the present study. When muscle cells are activated their membranes become more permeable to sodium (Nastuk & Hodgkin, 1950); exposure to potassium-rich media causes a temporary excitation and may conceivably lead to an increased intracellular concentration of sodium which in turn might stimulate respiration at a sodium-sensitive site.

In brain a correlation between the active ion transport and the rate of respiration has been suggested by Whittam (1962*a*). He compared the rates of oxygen uptake during incubation in 'balanced' and in sodium-deficient media and found that about 40 % of the oxygen consumption in a 'balanced' medium is caused by the active transport of sodium and potassium. However, he did not take into consideration that the respiration may not be equally well maintained in the different media. An increased rate of respiratory decline in the sodium-free medium would tend to increase the fraction of the total oxygen uptake that is due to the ion transport with the length of the incubation

period. In our experiments a sodium concentration of about 20 mM was required in the medium to maintain the respiration of rat-brain slices (Hertz & Schou, 1962); it is in qualitative agreement with Whittam's (1962*a*) data that the oxygen uptake in a medium with a sodium concentration of 20 mM is maintained at a level which is about 20% lower than the respiration in a medium with a sodium concentration of 125 mM.

No further increase in the respiration of brain slices is found when the sodium concentration in the medium is raised above 125 mM. The increased rate of oxygen uptake which brain slices show during incubation in potassium-rich media is accordingly also not due to a stimulation at a sodium-sensitive site but is probably caused by a direct stimulation at a potassium-sensitive site.

SUMMARY

1. Brain slices and muscle differ from other tissues (e.g. kidney and peripheral nerve) in showing an increased rate of respiration during incubation in media containing high concentrations of potassium.

2. The potassium-stimulated respiration declines rapidly both in muscle and in brain slices but follows a different course in each of the two preparations.

3. A minimum concentration of sodium in the medium is required to maintain the rate of oxygen uptake in brain slices and in muscle during prolonged incubation. Kidney slices, in contrast, are able to maintain their respiration in a sodium-free medium but at a decreased level.

4. An increased concentration of sodium in the medium causes a stimulated respiration in muscle but not in brain slices.

5. Within the central nervous system the effects of increased potassium concentrations or decreased sodium concentrations are limited to slices that contain grey matter from the brain. Neither white matter nor grey matter from the spinal cord shows an increased rate of oxygen uptake in potassium-rich media or a rapid respiratory decline in sodium-free media.

The authors thank the Common Cattle Market in Aarhus for its generous supply of calf brains; veterinarians J. Westh and C. J. Jensen for their help; Dr O. Perbøll (State Mental Hospital, Risskov) for the histological examinations; and Dr M. Schou for helpful discussions. The skilled technical assistance of Mrs J. Lyberth and Mrs I. Andresen is gratefully acknowledged.

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