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STUDIES OF THE RESPIRATION OF SYNAPTOSOMES

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Abstract—Synaptosomes were prepared from guinea pig cerebral cortex and their respiration studied under different conditions. Their respiratory rate of 15 μl O_2/mg protein/hr in phosphate buffer containing glucose agrees with published values. This rate was not altered by the addition of K^+, phenobarbitone, glutamic acid, glycine, γ-aminobutyric acid, acetyl choline, reserpine, nor-adrenaline, 5-hydroxytryptamine or histamine.

WHEN mammalian cerebral cortex is subjected to mild homogenisation, the synaptic complex seems to break off and reseal to form discrete particles which may be isolated by differential and density gradient centrifugation.[1–3] These particles were termed synaptosomes by Whittaker. They retain many of the constituents of the cell cytoplasm,[4] and also, many of the metabolic processes of intact cells.[5] Recent studies by Bradford[6] and Bradford and Thomas[7] have shown that synaptosomes are capable of quite substantial rates of respiration. There is evidence that synaptosomes can accumulate K^+[6,8] and it has been suggested that the synaptosomal membrane may be polarised and capable of being depolarised.[6,9] The respiration of isolated brain tissue can be altered markedly by some compounds influencing the membrane potential of cells,[10] and it has been reported that the same type of response can be detected in a preparation of synaptosomes.[9] In particular, substantial increases in the respiration of synaptosomes were obtained after the addition of K^+ to the preparation and by subjecting the synaptosomes to electrical pulses. In this study, we have determined the effects of a number of compounds on the respiration of synaptosomes, with special reference to those which affect synaptic membranes, and which might affect the degree of polarisation of the synaptosome membrane. The compounds tested include a number of possible transmitters of the central nervous system (acetylcholine, glutamic acid and the catecholamines), the membrane "stabiliser" phenobarbitone and the tranquiliser, reserpine. All of these compounds might be expected to affect synaptic membranes *in vivo*, and to alter the degree of polarisation of the synaptosomal membrane *in vitro* under certain circumstances.

MATERIALS AND METHODS

The isolation of synaptosomes was performed at 0–5°. Six-week old guinea-pigs were sacrificed by decapitation and their brains rapidly removed. The cortex was cut away from the rest of the brain, placed in 10% (w/v) 0·32 M sucrose and then homogenised in a teflon homogeniser running at 800 rev/min for 35 sec, during which

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time seven up and down strokes of the pestle were made. The suspension so formed was subjected to the fractionation procedure of Gray and Whittaker[2] as described and modified by Bradford.[6] This involved isolating the crude mitochondrial fraction by differential centrifugation and then isolating the enriched synaptosome band from a discontinuous sucrose gradient, consisting of equal volumes of 1·2 M and 0·8 M sucrose, which had been spun at 53,000 *g* for 2 hr. The synaptosome band lay between the 0·8 M and 1·2 M sucrose band.

The synaptosomes were resuspended in Krebs–Ringer phosphate solution[11] containing 11 mM glucose and 2 ml samples (containing approximately 7 mg of protein) were used for measurements of respiration. Reaction flasks, containing the 2 ml samples in the main compartment and 0·2 ml of 20% KOH in the centre wells were attached to a Gilson Differential Respirometer and gassed briefly with oxygen before the uptake of oxygen by the synaptosomes was measured, at 5-min intervals over a period of 20 min at 37°. The drug was then tipped into the main compartment and the measurements continued for a further 20 min.

Acetylcholinesterase (EC 3.1.1.7) activity was estimated, manometrically, by determining the release of CO_2 from acetylcholine perchlorate as described by Aldridge and Johnson.[12] Succinic dehydrogenase (EC 1.3.99.1) was estimated by the method of Quastel and Wheatley[13] using the modified medium described by Johnson and Whittaker.[4] Protein was estimated using the method of Gornal *et al.*,[14] using bovine serum albumin as standard. Electron microscopy was performed on samples of synaptosomes, using the procedure of Gray and Whittaker.[2]

RESULTS

The general properties of the synaptosomes were similar to those reported by other workers. Photographs taken under the electron microscope were very similar to those presented by Gray and Whittaker.[2] The ratio of acetylcholinesterase activity was 3:1 in favour of the synaptosomes compared with the mitochondria (Table 1), and this result is in agreement with that of De Robertis *et al.*[3] Three times as much succinic dehydrogenase activity was present in the mitochondrial fraction compared with the synaptosomal fraction (Table 1). If this result is expressed as a ratio of the total activity in each fraction, the ratio is 1·7:1 in favour of the mitochondrial fraction, a result which is in agreement with that of Johnson and Whittaker.[4] The ratio of the

TABLE 1. ACETYCHOLINESTERASE AND SUCCINIC DEHYDROGENASE ACTIVITIES OF THE SYNAPTOSOMAL AND MITOCHONDRIAL FRACTIONS OF FRESH GUINEA PIG BRAIN

	Series I		Series II	
Sample	Pr.	AChE activity units	Pr.	SDH activity units
syn	$4·1 \pm 0·1_{(3)}$	$148 \pm 2_{(3)}$	$2·5 \pm 0·1_{(3)}$	$119 \pm 7_{(3)}$
mit	$1·7 \pm 0·2_{(3)}$	$48 \pm 1_{(3)}$	$1·3 \pm 0·1_{(3)}$	$387 \pm 6_{(3)}$

Both the acetylcholinesterase (AChE) and the succinic dehydrogenase activity are expressed as μl of CO_2 evolved per mg protein per hour. The protein was estimated by the method of Gornal *et al.*

TABLE 2. THE EFFECT OF KCl PHENOBARBITONE ON THE RESPIRATION OF SYNAPTOSOMES

Flask contents main chamber	Soln. tipped	Rate before tipping μl O_2/mg Pr/hr	Rate after tipping μl O_2/mg Pr/hr
Suspension	KRP	15·3 ± 0·4	15·1 ± 0·3
Suspension	KCl	15·7 ± 0·9	15·0 ± 0·8
Suspension + Ph	KCl	14·9 ± 1·0	15·1 ± 0·9
Suspension	KRP	15·1 ± 0·6	15·3 ± 0·4
Suspension	Ph	14·5 ± 0·8	14·3 ± 1·0

The rates of respiration of the synaptosome samples were estimated as described in the text and are expressed as the means of five estimations ± S.E.M. The rates before and after tipping are statistically compared by Student's *t*-test.
Ph = Phenobarbitone (2 mM final conc.).
KRP = Krebs–Ringer phosphate.
KCl = 15 mM Potassium chloride (final conc.).

quantity of protein in each fraction (synaptosomes–mitochondria 2:1) is also in agreement with the results of Gray and Whittaker.[2] The oxygen utilisation, of approximately 15 μl O_2/mg Pr/hr (Table 2), was linear over the period 0–45 min at least (Fig. 1) and is in agreement with the results of Bradford.[6]

The addition of K^+, which stimulates oxygen consumption of cortex slices,[9] apparently by depolarising the cell membrane, was without effect upon the respiration of synaptosomes at a final concentration of 21·6 m-equiv./l. (Table 2). The same result was obtained for 106 m-equiv K^+/l. We had anticipated that, if K^+ was to depolarise

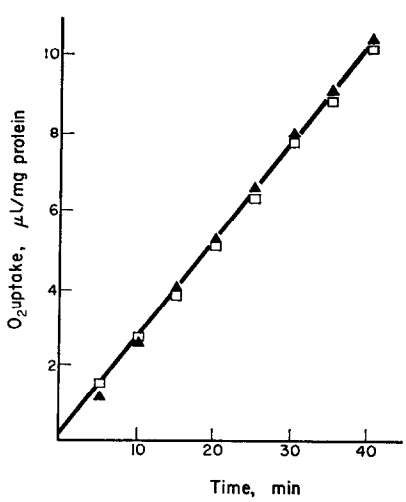

FIG. 1. The effect of the addition of KCl (15 mM) on the oxygen uptake of synaptosomes. The KC was tipped into synaptosomes whose respiratory rate in Krebs–Ringer phosphate solution had been estimated for 20 min. (▲) and the rate of oxygen uptake compared with controls (□). The details of the experimental procedure are given in the text. Each point is the mean of five estimations. (The S.E.M.s have been omitted since they were very small and of the same order as the symbols used.)

the synaptosomal membrane and increase the respiration, this effect might be prevented by the inclusion of phenobarbitone sodium, a membrane stabiliser,[15] in the incubation medium. This was not the case, however, and phenobarbitone sodium was, itself, without effect on the respiration of synaptosomes (Table 2).

L-glutamate, an amino acid which may be implicated in synaptic transmission in the CNS,[16] has already been shown not to alter the respiratory rate of synaptosomes appreciably.[6,7] In our experiments, L-glutamate (10 mM) caused a slight lowering of the respiratory rate, but the effect was not significant (Table 3), so that the linearity of the respiratory rate was not altered.

TABLE 3. THE EFFECT OF L-GLUTAMATE, GABA, GLYCINE ACETYLCHOLINE, RESERPINE, *n*-ADRENALIN, 5-HYDROXYTRYPTAMINE AND HISTAMINE ON THE RESPIRATION OF SYNAPTOSOMES

Soln. tipped	Rate before tipping (μl O_2/mg Pr/hr)	Rate after tipping (μl O_2/mg Pr/hr)
KRP	21·0 ± 0·9	20·6 ± 0·5
glut	21·6 ± 0·2	20·1 ± 1·0
KRP	17·1 ± 1·3	17·0 ± 1·3
GABA	16·6 ± 0·8	17·3 ± 1·2
glycine	15·8 ± 2·1	15·8 ± 1·8
ACh	15·8 ± 0·5	16·1 ± 0·2
Res	19·4 ± 2·9	17·6 ± 2·6
KRP	17·4 ± 0·8	15·6 ± 1·2
SHT	16·2 ± 1·0	15·1 ± 1·0
Hist	16·0 ± 1·2	15·0 ± 0·7
n-A	15·8 ± 1·1	15·4 ± 0·8

The rates of respiration of the synaptosome samples were estimated over a 20-min period as described in the text, and the results are expressed as the means for four estimations (three for glut) ± S.E.M. The results before and after tipping are statistically compared by the Student's *t*-test, but no significant difference was found.

KRP = Krebs–Ringer phosphate (controls).
glut = L-Glutamic acid (10 mM final conc.).
GABA = γ Amino butyric acid (5 mM final conc.).
glycine = (2 mM final conc.).
Res = Reserpine (8·3 μg/ml final conc.).
ACh = Acetylcholine (250 μM final conc.).
SHT = 5-Hydroxytryptamine (20 μM final conc.).
Hist = Histamine (20 μM final conc.).
n-A = nor-Adrenalin (20 μM final conc.).

In the next series of experiments, compounds, which have been reported in the literature to affect polarised membranes, but which do not appear to have been examined for their effect on the respiration of synaptosomes, were tested. These were acetylcholine, noradrenalin and 5-hydroxytryptamine which have all been reported as possible transmitters at CNS synapses,[16] and γ amino butyric acid, glycine and histamine all of which have been proposed as possible inhibitory transmitters of the

CNS.[16] The tranquilising drug, reserpine, which is reported to cause the release of 5-hydroxytryptamine and the catecholamines[17] was also tested. Table 3 shows that none of these compounds had any effect on the respiration (the linearities of the O_2-uptake/time were not altered), and the oxygen uptakes measured only 2 min after the addition of the compounds to the synaptosomes agreed with this conclusion.

DISCUSSION

The characteristics, so far reported, for the preparations of synaptosomes suggest that the particle, itself, might be used as a model for studying the effects of drugs on the neuron. This possibility is of some importance because of the comparative ease with which synaptosomes can be prepared and the difficulties of preparing neurons retaining structural integrity.[18] However, the results obtained here suggest that synaptosomes, whilst retaining certain properties of excitable (polarised) cells, as reported by others (the K^+ gradient for example), do not respond in the manner one might expect, to compounds reported to alter the membrane potential of cells. None of the conditions tested significantly altered the respiration of synaptosomes. This result was not in agreement with results reported by Bradford[9] who found that the rate of respiration of synaptosomes could be increased by increasing the K^+ concentration of the medium to 55 m-equiv./l., a response which also occurs in cerebral cortex slices when they are treated in the same way. It is possible that in our experiments, the rate of respiration might be limited by the rate of diffusion of oxygen through the medium. We have found, however, if twice the amount of synaptosomes was resuspended in the same volume of medium, twice the volume of oxygen was absorbed. It is, therefore, unlikely that the rate of diffusion of oxygen is a limiting factor to synaptosomal respiration. We can offer no reason for the difference between our result and that of Bradford, since, in all other respects (appearance and enzymic activities) our preparation was very similar to those of other workers.

Our preparation should contain a heterogeneous mixture of nerve-ending types[3] and it is difficult to understand, therefore, why none of the transmitters tested altered the respiratory rate, if this rate is coupled to the state of the membrane as in cerebral cortex slices. Although acetycholine may be a transmitter at only 15 per cent of cortex synapses,[19] the proposed presynaptic role of this compound might also be significant here, since the synaptosomal membrane is, essentially the presynaptic membrane. Koelle[20] has proposed that, upon the arrival of an impulse at some cholinergic synapses, the acetycholine first released binds to the presynaptic membrane to cause the release of more of the same transmitter, or indeed, of another transmitter.[16,21] This could explain the wide distribution of acetylcholine and acetylcholinesterase in the cortex and the apparent low concentration of cholinergic synapses. Thus, if the theory is true, one might expect acetylcholine to affect the respiration of the synaptosomes, even if the other transmitters are without effect because they predominantly act on the post-synaptic membrane. On the other hand the results could be explained if the synaptosome membrane is not polarised, or if it is polarised it is insensitive to the compounds tested under our experimental conditions. Another possible explanation of the negative results is that the changes in the polarised state of the membrane are not sensitively geared to the gross respiratory rate as we measure it.

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