STIMULATION OF BRAIN RESPIRATION BY THIOSALICYLIC ACID

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The intermediary metabolism of brain cortex slices is known to be sensitive to changes in the potassium ion concentration of the medium. The activating effects of potassium ions were first studied by Dickens and Greville (1935) and Ashford and Dixon (1935), and appeared to be identical to those due to an oscillating electric potential. The potassium-stimulated respiration was inhibited by malonic acid and this inhibition was reversed by oxaloacetic acid and only to a small extent by fumaric acid (Parmar and Quastel, in preparation), suggesting participation of the citric acid cycle in the stimulated brain respiration. Our results are consistent with the fact that the rate of formation of $C^{14}O_2$ during oxidation of glucose-U-C^{14} and fructose-U-C^{14} is greatly increased by the presence of potassium ions.

The present paper reports stimulation of the respiratory activity of rat brain cortex slices by thiosalicylic acid (TSA). This stimulation was accompanied by a decrease in the production of lactic acid and differed from that produced by potassium chloride, where the increase of lactic acid paralleled enhanced oxygen uptake and substrate utilization. The parent compound, salicylic acid, has been found to increase the oxygen uptake of brain cortex slices (Fishgold et al., 1951) and is capable of uncoupling phosphorylation from oxidation (Brody, 1956). In recent studies we have found that salicylic acid also produces a noncompetitive inhibition of purified mitochondrial succinic dehydrogenase (Hellerman et al., 1960).

Experimental

The rats (Body weight 200-250 g.) were killed by decapitation. The brains were removed and placed in ice cold phosphate-Ringer solution contain-

ing: NaCl, 128 mM; KCl, 5 mM; $CaCl_2$, 0.6 mM; $MgSO_4$, 1.3 mM; sodium phosphate buffer at pH 7.4, 10 mM. Substrate, when added, was glucose (10 mM) or sodium pyruvate (10 mM). Slices of rat brain cortex were cut with a Stadie-Riggs slicer. The slices were weighed wet immediately and their dry weight calculated by a factor determined by drying a known quantity of wet tissue to constant weight at 110°C. All incubations were carried out in a conventional Warburg manometric apparatus at 37°C in Krebs-Ringer phosphate solution with oxygen as the gas phase. In all experiments the vessels were gassed for 5 minutes with thermal equilibration of 10 minutes. The central well contained 0.2 ml of 20% KOH. Potassium chloride and TSA were present in the side arm and were tipped into the main vessel after thermal equilibration. At the end of the incubation period, 3 ml of 30% TCA was added to each vessel and the deproteinized supernatant was used for chemical analyses. Glucose utilization was estimated by using aminobiphenyl (Timell et al., 1956), lactic acid and pyruvic acid by the methods of Barker and Summerson(1941) and Friedemann and Haugen (1943) respectively.

Results and Discussion

It is well known that glucose is the major metabolic substrate for nervous tissue, where it is believed to be metabolized aerobically through the glycolytic or Embden-Meyerhof pathway and the citric acid cycle.Typical effects of TSA on oxygen uptake, lactic and pyruvic acid production, and glucose utilization in the absence and presence of potassium chloride are shown in Table I. TSA and potassium chloride increased oxygen uptake and glucose utilization. The rate of oxygen uptake was decreased by concentrations of TSA above 1 mM. TSA decreased lactic acid production, but did not prevent the increase due to potassium chloride. The lactic acid produced under these conditions is derived via the Embden-Meyerhof pathway of glucose catabolism.The loss of aerobic glycolytic activity of the rat brain cortex slices in the presence of TSA may be assumed to be connected with a loss of potassium ions since lactate formation was increased when both TSA and potassium chloride were present in the medium. This can be compared with the effect of glutamate which

Table I

Metabolism of Glucose in Rat Brain Slices

Additions	Oxygen* Uptake A	Glucose Utilized B	Lactate Formed C	Pyruvate Formed D	Glucose Oxidised E $(B-\frac{C+D}{2})$	Ratio : $\frac{A}{E}$
Nil	63±6(5)	21±2(5)	18±2(5)	1.4±.2(4)	11.3	5.7
KCl	105±6(5)	60±2(5)	74±3(5)	1.2±.2(4)	22.4	4.7
TSA (0.1 mM)	96±4(5)	37±2(5)	11±2(5)	1.4±.2(4)	30.8	3.1
TSA (0.1 mM) + KCl	114±5(6)	76±2(6)	77±2(6)	1.2±.2(4)	36.9	3.1
TSA (0.25 mM)	120±5(4)	50±2(4)	4±2(4)	1.4±.2(4)	47.3	2.6
TSA (0.25 mM) + KCl	131±4(4)	78±2(4)	75±3(4)	1.2±.3(4)	39.9	3.3

* Corrected for endogenous oxygen uptake.
Substrate: glucose (10 mM). Medium: Krebs-Ringer phosphate solution, pH 7.4. KCl: 100 mM. All results obtained were expressed as μ Moles per 100 mg (dry weight) of slices per 90 minutes. Mean and Standard deviations are given. Number of experiments were shown in parentheses in the Tables.

was shown by Weil-Malherbe (1938) to restore aerobic glycolysis, and suggests that the latter effect also may be connected with the maintenance or restoration of the potassium content of the slice.

Further, the ratio of oxygen uptake to glucose oxidation was found to be 5.7, which approaches the approximate theoretical value of 6, assuming complete oxidation of glucose to carbon dioxide and water. This value was only slightly reduced during potassium stimulation, where a ratio of 4.7 was found. In previous studies Quastel (1958) has reported values of approximately 6 for the ratio in potassium-stimulated glucose oxidation. In our studies the ratio was reduced to approximately 3 by TSA, both in the presence and absence of potassium chloride. This ratio indicates less complete oxidation of glucose

in the presence of TSA. Stimulation by this agent thus differs from that due to potassium chloride, where increased glucose utilization is approximately accounted for by complete oxidation to carbon dioxide and water. It may be assumed from these data that TSA accelerates biochemical processes whereby glucose is converted into various amino and fatty acids. However, more experimental evidence is needed before the validity of this interpretation of the effects of TSA can be assured.

The effects of TSA on pyruvate metabolism in brain cortex slices are shown in Table II. Pyruvate, like glucose, maintained oxygen uptake at a fairly high level. TSA and potassium chloride caused an increase in both oxy-

Table II

Pyruvate Oxidation in Rat Brain Cortex Slices

Additions	Oxygen* Uptake A	Pyruvate Utilized B	Lactate Formed C	Pyruvate Oxidised D (B-C)	Ratio: $\frac{A}{D}$
Nil	54±4(4)	53±2(4)	25±2(4)	28	1.9
KCl	99±4(4)	68±3(4)	16±2(4)	52	1.9
TSA (25 mM)	90±3(4)	68±2(4)	23±2(4)	45	2.0
TSA (25 mM) + KCl	118±4(4)	78±3(4)	20±3(4)	58	2.0

* Corrected for endogenous oxygen uptake.
Substrate: sodium pyruvate (10 mM). Medium: Krebs-Ringer phosphate solution, pH 7.4. KCl: 100 mM.

gen uptake and pyruvate utilization. The production of lactic acid was not altered under these conditions. In addition, the ratio of oxygen uptake to pyruvate utilized was found to be approximately 2, both in the presence and absence of TSA and/or potassium chloride. Complete oxidation to carbon dioxide and water requires 5 atoms of oxygen per mole of pyruvic acid. The ratio of 2 obtained in our experiments as compared to the theoretical ratio of 2.5 may be within the experimental error. A similar ratio for the oxidation of

pyruvic acid was reported by Montgomery and Webb (1956), using heart mitochondria.

From these considerations it can be concluded that glucose is metabolized aerobically through the glycolytic and citric acid cycles in brain slices. The presence of TSA or potassium chloride stimulates respiration and the oxidation of both glucose and pyruvate. Further experimental evidence is required to elucidate the mechanism by which aerobic glycolysis is inhibited by TSA and restored by potassium chloride.

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