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## Effect of cinnamic acid on potassium stimulated respiration in rat brain cortex slices

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IT IS well established that most of the energy requirements of brain are satisfied by respiration and glycolysis.[1] Glucose is the preferred substrate for brain respiration in comparison to the other substrates which, though utilized, have been shown to sustain less stable respiration.[2] Changes in potassium ion concentration have a profound effect on the intermediary metabolism of brain cortex slices. Increase in the concentration of potassium ions has been shown to result in a simultaneous increase in the oxygen uptake by respiring cerebral cortex slices.[3, 4] Similar effects were observed by applying electrical impulses.[5] On the other hand, similar stimulation of the respiratory activity of rat brain cortex slices by thiosalicylic acid was found to be accompanied by a decrease in the production of lactic acid[6] and thus differed from that produced by potassium chloride where increase in lactic acid paralleled enhanced oxygen uptake and substrate utilization.[7] In the present study evidence has been provided that cinnamic acid, shown to interfere in cellular metabolism,[8] caused supression of the potassium activated respiratory carbon dioxide with little or no inhibitory effects on the unstimulated processes during oxidation of glucose and L-glutamate by rat brain cortex slices.

All the experiments were carried out on brain cortex slices from rats of either sex, weighing about 200–250 g. The rats were killed by decapitation. The brains were removed and placed in ice cold phosphate–Ringer solution containing 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. The substrates used were either glucose (10 mM) or L-glutamate (10 mM). Rat brain cortex slices were cut with a Stadie–Riggs tissue slicer and were weighed immediately. The dry weight of the slices was calculated by a factor determined by drying a known quantity of wet tissue to constant weight at 110°. The slices were incubated in a conventional Warburg Manometric apparatus at 37° in Krebs–Ringer phosphate solution using oxygen as the gas phase. The vessels were gassed for 5 min and were equilibrated for further 10 min. The central well contained 0·2 ml of 20% KOH. Potassium chloride and cinnamic acid were present in the side arm and were tipped in the main vessel after thermal equilibration.

The values for the oxygen uptake after the addition of potassium chloride (100 mM) and the effect of cinnamic acid during oxidation of glucose or sodium glutamate in the presence and absence of potassium chloride are indicated in Table 1. Our results are in good agreement with those of other investigators,[3, 4] where a definite increase in the oxygen uptake was observed after addition of potassium chloride (100 mM) during oxidation of glucose and L-glutamate by rat brain cortex slices.

TABLE 1. EFFECT OF CINNAMIC ACID ON OXIDATION OF GLUCOSE AND L-GLUTAMATE BY RAT BRAIN CORTEX SLICES

| Additions | $Q_{O_2}$ Values | | | |
|---|---|---|---|---|
| | 0–30 min | | 30–60 min | |
| | −KCl | +KCl | −KCl | +KCl |
| Glucose | 12·37 ±0·23 | 22·27 ±0·66 | 11·62 ±0·26 | 20·45 ±0·34 |
| Glucose + cinnamic acid | 11·47 ±0·28 | 12·40 ±0·22 | 11·25 ±0·24 | 11·30 ±0·27 |
| L-Glutamate | 13·92 ±0·18 | 17·72 ±0·28 | 11·65 ±0·17 | 14·65 ±0·21 |
| L-Glutamate + cinnamic acid | 13·22 ±0·22 | 13·10 ±0·28 | 11·27 ±0·17 | 11·07 ±0·11 |

All values are mean of four experiments. The $Q_{O_2}$ values with standard error are calculated on the basis of dry weight of the rat brain cortex slices as indicated in the text. The final concentrations of glucose and L-glutamate were 10 mM, those of cinnamic acid and KCl were 5 mM and 100 mM respectively. The vessel contents and assay procedure are as mentioned in the text.

The increased respiratory activity was found to remain uniform for a period of 1 hr. Administration of cinnamic acid at a final concentration of 5 mM was found to inhibit the potassium stimulated respiration with a consequent decrease in the oxygen uptake observable during oxidation of both glucose as well as L-glutamate. Cinnamic acid in the above mentioned concentration was found to have no appreciable effect on the unstimulated respiration. Further increase in the concentration of cinnamic acid inhibited both the stimulated as well as the unstimulated respiration. Cinnamic acid has also been reported to inhibit butyrate oxidation in slices of guinea pig kidney[9, 10] and production of ammonia in rat kidney slices in the presence of glucose.[11] In the present study evidence has been presented for interference by cinnamic acid in the reactions of tricarboxylic acid cycle since stimulatory effects of potassium chloride are concerned with the activation of citric acid cycle.[7, 12] It could be presumed that cinnamic acid inhibits the ATP formation in the neurone associated with the oxidation of glucose and L-glutamate through the citric acid cycle. However, further experimental evidence is needed to elucidate the inhibition of potassium stimulated respiration by cinnamic acid in rat brain cortex slices.

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## The effect of Flagyl on xanthine oxidase and alcohol dehydrogenase*

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FLAGYL (metronidazole†), a drug used to combat trichomonal infection of the vagina, has recently been shown to produce aversion to ethanol. Because of the latter effect, the drug is being tested as a repessant of compulsive consumption of alcohol.[1] The mechanism of this antagonism between ethanol and metronidazole is not yet known.

A similar ethanol antagonism is produced by disulfiram (Antabuse). Disulfiram has been shown to inhibit several enzymes related to alcohol metabolism,[2] but the principal effect is attributed to the blocking of acetaldehyde oxidation. One of the enzymes inhibited by disulfiram, which are involved in the oxidation of acetaldehyde, is xanthine oxidase.[3] As part of a survey of the mechanism of action of Flagyl, its effect on purified milk and liver xanthine oxidase (1.2.3.2) and on liver alcohol dehydrogenase (1.1.1.1) was tested.

### METHODS

Cream xanthine oxidase and horse liver alcohol dehydrogenase were obtained from Worthington (Freehold, N.J.) and were used without further purification. Liver xanthine oxidase was prepared from the supernatant fraction of rat liver by a modification of a method previously described for beef liver[4] and was purified by ammonium sulfate fractionation; the fraction precipitated between 20 and 50 per cent saturation was used as enzyme source. Flagyl was dissolved in 0·1 M phosphate, pH 7·8, at the concentration of 0·01 M. Xanthine oxidase activity was assayed spectrophotometrically by uric acid formation, at 298 mμ,[5] and xanthine dehydrogenase activity colorimetrically by reduction of nitro-BT tetrazolium salt, in the presence of phenazine-methosulfate and gelatin at 540 mμ.[6] Fine chemicals were obtained from Sigma (St. Louis). The reaction mixture, without xanthine, was pre-incubated for 5 min at 25°, and the reaction was started by the addition of xanthine. The reaction rate

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† Flagyl: 1-(2′-hydroxyethyl)-2-methyl-5-nitroimidazole.