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Effect of 2-methyl-3-ortho-tolyl-4-quinazolone on the oxidation of pyruvic acid in brain

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AT PRESENT little is known about the biochemical changes in the central nervous system induced by anticonvulsant drugs of the quinazolone series. In an attempt to gain information on the mechanism of action of these drugs, the effect of 2-methyl-3-ortho-tolyl-4-quinazolone (QZ-2) was investigated on the oxidation of pyruvic acid, using rat brain homogenate and isolated brain mitochondria. Apart from its anticonvulsant property[1] QZ-2 has been found to be a potent hypnotic and a central nervous system depressant.[2] Furthermore, inhibition of potassium-stimulated oxidation of glucose in slices of rat cerebral cortex at pharmacological concentrations of QZ-2 (Parmar *et al.*, in preparation) accounts for its activity towards the tricarboxylic acid cycle. In the present investigation QZ-2 has been found to depress the oxidation of pyruvic acid in brain tissue. This inhibition was considerably decreased by the addition of diphosphopyridinenucleotide (DPN).

QZ-2 was prepared in our laboratory by the method reported by Kacker and Zaheer.[3] Brain mitochondria were isolated by differential centrifugation of 10% (w/v) rat brain homogenate in cold 0·25 M sucrose as described earlier.[4] All incubations were carried out in the conventional Warburg manometric apparatus at 37° with air as the gas phase. The central well contained 0·2 ml of 20% KOH. The vessel contents were the same as described by Beer and Quastel.[5] In some experiments pyruvic acid was estimated[6] after 90 min in the deproteinized (3 ml 30% trichloroacetic acid) supernatant.

As shown in Table 1, QZ-2 at 1 mM concentration caused significant inhibition of pyruvic acid oxidation in brain homogenate. Oxygen uptake was decreased in the first 30 min and the degree of

TABLE 1. EFFECT OF QZ-2 ON PYRUVATE OXIDATION BY RAT BRAIN HOMOGENATE

Additions	Inhibition (%)			
	0–30 min		30–60 min	
	−DPN	+DPN	−DPN	+DPN
QZ-2 (1 mM)	38·56 ± 0·51	15·2 ± 0·32	36·18 ± 0·25	Nil
QZ-2 (2 mM)	50·1 ± 0·15	18·4 ± 0·11	46·4 ± 0·25	Nil
QZ-2 (3 mM)	61·02 ± 0·39		59·3 ± 0·38	

All values are mean of five experiments. The oxygen uptake in control experiments was 48·4–60·9 μl and 35·3–59·1 μl in the absence of DPN and 52·5–72·5 μl and 36·3–59·1 μl in the presence of DPN during first 30 min and the next 30–60 min respectively. Percent inhibition and standard error are calculated from oxygen uptake per 400 mg wet weight. Final concentrations of pyruvic acid and DPN were 10 mM and 0·25 mM respectively.

inhibition remained more or less constant for subsequent 30 min. Increase in the concentration of QZ-2 to 2 mM and 3 mM caused progressive increase in the inhibition of pyruvate oxidation. Addition of DPN slightly increased the respiratory acitvity. It was interesting to find that the addition of DPN restored the activity of brain homogenate inhibited by QZ-2 in these experiments. In the presence of DPN no inhibition was observed with 1 mM of QZ-2 in the 30- to 60-min period during oxidation of pyruvic acid.

The inhibitory effects of QZ-2 on the rate of oxidation of pyruvic acid by rat brain mitochondria during 30-, 60- and 90-min periods is shown in Table 2. No significant increase in the oxygen uptake was observed in the presence of DPN. Inhibition of the oxygen uptake progressively increased with increasing concentrations of QZ-2. The presence of DPN caused similar protection

homogenate. These results point out the participation of DPN as a cofactor where QZ-2 might be acting by interfering with DPN. Our results in some way parallel the findings of Beer and Quastel[7] where inhibition by acetaldehyde during pyruvate oxidation by brain mitochondria was completely abolished by the addition of DPN. Such restoration of enzyme activity was not significant in the last 60- to 90-min period in spite of the presence of DPN. It may be assumed that DPN might have

TABLE 2. EFFECT OF QZ-2 ON PYRUVATE OXIDATION BY RAT BRAIN MITOCHONDRIA

Additions	Percent Inhibition							
	Oxygen uptake						Pyruvate utilization	
	0–30 min		30–60 min		60–90 min		0–90 min	
	−DPN	+DPN	−DPN	+ DPN	−DPN	+DPN	−DPN	+DPN
QZ-2 (1 mM)	22·3 ±0·28	8·13 ±0·13	20·65 ±0·26	8·2 ±0·14	20·7 ±0·27	15·2 ±0·25	22·15 ±0·5	8·3 ±0·09
QZ-2 (2 mM)	35·55 ±0·13	29·6 ±0·25	29·1 ±0·29	24·4 ±0·2	39·2 ±0·26	32·0 ±0·02	39·1 ±0·08	13·1 ±0·12
QZ-2 (3 mM)	51·1 ±0·16		55·3 ±0·18		54·3 ±0·13		61·5 ±0·24	

All values are mean of five experiments. The oxygen uptake in the control experiments was 30·5–45·6 μl, 35·3–53·3 μl and 36·1–55·1 μl in the absence and 35·2–51·5 μl, 35·6–52·8 μl and 33·3–55·4 μ in the presence of DPN during first 30 min, 30–60 min and 60–90 min respectively. Pyruvate utilization in the control experiments after 90 min was 8·41–12·2 μmoles in the absence of DPN and 10·9–15·2 μmoles in the presence of DPN. Percent inhibition and standard error are calculated from oxygen uptake and pyruvate utilization per 400 mg wet weight. Final concentrations of pyruvic acid and DPN were 10 mM and 0·25 mM respectively.

against the inhibitory effects by QZ-2 as was observed during pyruvate oxidation by brain been utilized during oxidation in a one-hour period. Pyruvic acid utilization was similarly inhibited progressively with increase in the concentration of QZ-2. This utilization of pyruvic acid was greater in the presence of DPN indicating the restoration of the activity of enzymes inhibited by QZ-2.

In conclusion QZ-2 inhibits the oxidation of pyruvic acid in rat brain homogenate and isolated mitochondria presumably by competing with DPN. Further investigations may elucidate the biochemical mechanism of QZ-2 and other anticonvulsant drugs.

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REFERENCES

1. M. L. GUJRAL, K. N. SAREEN and R. P. KOHLI. *Indian J. med. Res.* **45**, 207 (1957).
2. M. L. GUJRAL, P. N. SAXENA and R. S. TEWARI. *Indian J. med. Res.* **43**, 637 (1955).
3. I. K. KACKER and S. H. ZAHEER. *J. Indian chem. Soc.* **28**, 344 (1951).
4. S. S. PARMAR, M. C. SUTTER and M. NICKERSON. *Canad. J. Biochem. Physiol.* **39**, 1335 (1961).
5. C. T. BEER and J. H. QUASTEL. *Canad. J. Biochem. Physiol.* **36**, 543 (1958).
6. S. REITMAN and S. FRANKEL. *Amer. J. Clin. Path.* **28**, 56 (1957).
7. C. T. BEER and J. H. QUASTEL. *Canad. J. Biochem. Physiol.* **36**, 531 (1958).