The Influence of Perathiepine and Chlorpromazine on Some Enzyme Reactions in Rat Brain Preparations

Perathiepine [10-(4-methylpiperazino)-10,11-dihydrodibenzo(b,f)thiepine] was synthesized by JÍLEK et al.[1] and was shown in pharmacological examination to be a potent thymoleptic drug[2]. Although the exact mechanism of action of psychotropic drugs is not fully understood as yet, it may be assumed that their influence on various enzyme reactions of the central nervous system plays an important role. It was therefore of interest to study the influence of perathiepine upon the oxidation of pyruvate, oxoglutarate, and succinate, on 2 enzymes involved in glycolysis (hexokinase and glucoso-6-phosphatase), and finally on Mg^{++}-activated, DNP-activated, and $Mg^{++}Na^{+}$-activated, K^{+}-stimulated adenosinetriphosphatase. The results were then compared with the action of a known thymoleptic chlorpromazine.

In all experiments, male Wistar rats weighing 170–200 g were used. After killing, the brains were removed as soon as possible. The tissue was disintegrated in an Elvhjem-Potter homogenizer in 0.3 *M* sucrose at 2–4 °C. In some experiments, as indicated below, the mitochondrial fraction prepared according to ALDRIDGE[3] or the 10,000 *g* supernatant were used. The consumption of oxygen was followed with the conventional Warburg technique on air. The protein content was determined by nesslerization, the inorganic phosphate by the method of TAUSSKY and SHORR[4], and glucose after HUGGETT and NIXON[5]. The composition of incubation media is given in the Tables.

As may be seen in Table I, chlorpromazine up to the concentration of 1.0 m*M* has practically no effect on the endogenous respiration and oxidation of oxoglutarate and succinate. On the other hand, the addition of pyruvate plus malate to the medium containing 1.0 m*M* chlorpromazine or more does not cause any increase in oxygen consumption, indicating that the oxidation of this substrate is completely blocked. Similar results were obtained when perathiepine was used as inhibitor, and pyruvate plus malate as substrates. The inhibition of oxidation of other substrates starts at lower concentrations, but at

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Table I. Influence of perathiepine and chlorpromazine on the oxidation of pyruvate, oxoglutarate, and succinate in brain homogenates

Substrate	% inhibition[a]					
	Perathiepine			Chlorpromazine		
	0.2 m*M*	1.0 m*M*	2.0 m*M*	0.2 m*M*	1.0 m*M*	2.0 m*M*
Pyruvate + malate	2	32	87	9	47	84
Oxoglutarate	11	7	48	5	38	62
Succinate	− 1	1	35	− 4	8	46
Endogenous respiration	1	6	76	− 1	29	69

[a] Medium: 20 m*M* K-phosphate buffer pH 7.2, $MgSO_4$ 8 m*M*, sucrose 86 m*M*, ATP 1 m*M*, DPN 1 m*M*, nicotinamide 40 m*M*, KCl 62.5 m*M*, homogenate 1 ml, substrates 10 m*M*; total volume 3.5 ml. Incubation 60 min at 30 °C. Average values from 2–12 experiments.

Table II. Influence of perathiepine and chlorpromazine on enzyme reactions

Enzyme	% inhibition[a]							
	Perathiepine				Chlorpromazine			
	0.05 m*M*	0.1 m*M*	0.2 m*M*	0.4 m*M*	0.05 m*M*	0.1 m*M*	0.2 m*M*	0.4 m*M*
Mg^{++}-activated ATPase in mitochondria[b]	3	27	37	43	− 4	23	36	47
Mg^{++}-activated ATPase in 10,000 *g* supernatant[c]	4	10	15	48	− 4	10	19	30
DNP-stimulated ATPase[d]	32	39	44	47	17	24	24	29
NaKA[e]	31	48	74	87	45	54	100	100
Hexokinase[f]	− 3	0	4	0	25	25	18	25
Glucoso-6-phosphatase[g]	8	5	5	5	8	−4	−4	12

[a] Average values from 2–4 experiments. [b] Medium: *Tris*-HCl buffer, pH 7.4, 40 μM; $MgSO_4$ 8 μM; KCl 90 μM; ATP 0.5 μM; sucrose 60 μM; mitochondrial suspension 0.2 ml in 1.0 ml. Incubated 30 min at 30 °C. [c] Medium: *Tris*-HCl buffer, pH 7.4, 40 μM; $MgSO_4$ 6 μM; sucrose 270 μM; supernatant 0.2 ml in 1.0 ml. Incubated 30 min at 30 °C. [d] Medium: *Tris*-HCl buffer, pH 7.4, 40 μM; DNP 0.1 μM; KCl 90 μM; ATP 0.5 μM; sucrose 60 μM; mitochondrial suspension 0.2 ml in 1.0 ml. Incubated 30 min at 30 °C. [e] Medium A: *Tris*-HCl buffer, pH 7.4, 40 μM; $MgSO_4$ 6 μM; NaCl 100 μM; KCl 20 μM; ATP 2 μM; sucrose 30 μM; supernatant 0.2 ml in 1.0 ml. Medium B: medium A but without NaCl, KCl, and with sucrose 270 μM. Activity: A minus B. Incubated 30 min az 30 °C. [f] Medium: *Tris*-HCl buffer, pH 7.4, 40 μM; glucose 2 μM; ATP 1 μM; $MgSO_4$ 8 μM; KCl 90 μM; sucrose 60 μM; homogenate 0.2 ml in 1.0 ml. Incubated 30 min at 30 °C. [g] Medium: Citrate buffer, pH 6.5, 40 μM; glucoso-6-phosphate 6 μM; KCl 90 μM; sucrose 60 μM; homogenate 0.2 ml in 1.0 ml. Incubated 15 min at 30 °C.

2.0 mM there are only small differences in the degree of inhibition caused by both drugs.

The action of perathiepine and chlorpromazine on various enzyme systems is summarized in Table II. As can be seen, both drugs have only little effect on the rat brain hexokinase and glucoso-6-phosphatase. The influence of both drugs on the Mg^{++}-activated adenosinetriphosphatase in brain mitochondria and in 10,000 g supernatant is in general of a similar degree. In contrast to this, perathiepine seems to be a more potent inhibitor of the enzyme contained in 10,000 g supernatant.

Similarly, perathiepine is evidently a stronger inhibitor of brain mitochondrial 2,4-dinitrophenol stimulated adenosinetriphosphatase. Finally, the influence of perathiepine and chlorpromazine on the $Mg^{++}Na^{+}$-activated, K^{+}-stimulated adenosinetriphosphatase in 10,000 g supernatant (so-called 'NaKA'[6-8]) was studied. This enzyme differs from other adenosinetriphosphatases by its organ and cellular localization and by the sensitivity to strophantine and various cations[9,10]. NaKA is an essential constituent of the sodium pump, a system responsible for the transport of sodium and potassium across the cell membrane against the concentration gradient, which has a decisive importance for the excitability of the nervous system[11].

The activity of this enzyme is completely inhibited by chlorpromazine at 0.2 mM and more. The inhibition caused by perathiepine, although also very strong, does not reach the degree of the latter drug, and even at 0.4 mM about 10% of initial activity is detectable.

It may be concluded that, regardless of some minor quantitative differences, the action of both drugs upon the reactions studied here is in general of similar nature. The most outstanding features of this action are (1) relative ineffectiveness towards glycolytic enzymes, (2) inhibition of the oxidation of pyruvate, (3) strong inhibition of NaKA. This last effect may be considered as most important for pharmacology.

Zusammenfassung. Die Wirkung des Thymolepticums Prothiaden [10-(4-Methylpiperazino)-10,11-dihydrobenzo(b,f)thiepin] auf die Oxydation des Pyruvats, Oxoglutarats und Succinats sowie auf die Aktivität der Hexokinase, Glucoso-6-phosphatase und Mg^{++}-aktivierten, DNP-aktivierten und $Mg^{++}Na^{+}$-aktivierten, K^{+}-stimulierten Adenosintriphosphatase (NaKA) wurde untersucht und mit der Wirkung von Chlorpromazin verglichen. Im allgemeinen weisen beide Substanzen ähnliche Eigenschaften auf, welche in einer relativen Unwirksamkeit gegenüber glykolytischen Enzymen, Hemmung der Pyruvatoxydation und starker NaKA-Hemmung bestehen.

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Catalysis and Inhibition of the Oxidative Degradation of Deoxyribonucleic Acid by Catalase Showing Maximum Catalytic Effect in Pico-Molar Concentrations

As is known, catalase not only decomposes hydrogen peroxide but it can also exert a peroxidatic function with certain acceptors when hydrogen peroxide is released slowly[1,2]. It can further act as a non-specific hematin catalyst[3]. The experimental results presented in the following give evidence that the oxidative degradation of deoxyribonucleic acid (DNA) is catalysed by very low concentrations of catalase. When the concentration of catalase is increased, the catalytic effect decreases and is even inverted into a protective effect.

The experimental conditions and analytical methods used have been described before[4]. The DNA used contained about 0.01% iron. The solution of DNA was prepared with 10% aqueous sodium chloride containing 0.033 M/l phosphate buffer, pH 7, and 0.002 M/l sodium pyrophosphate. The specific viscosity of the 0.1% DNA solution was 0.71. Catalase was from Boehringer (Mannheim) and ferrichloride from Merck (Darmstadt), both of analytical grade. The experiments and the viscosity measurements were carried out at 37 °C.

Figure 1 shows the specific viscosity of the DNA solution after 1000 h storage under oxygen with the addition of different amounts of catalase or of ferrichloride. The results indicate that there is a maximum decrease of the viscosity when the solution contains $2 \cdot 10^{-12}$ M/l of catalase. Surprisingly, the catalytic effect becomes smaller not only with decreasing but also with increasing catalase concentrations and the effect almost disappears when 10^{-6} M/l or more of catalase are present. A similar type of curve is obtained when iron-III-chloride is added in place of catalase, although the effect is substantially smaller. Again, the maximum effect is obtained with about $2 \cdot 10^{-12}$ M/l of the additive.

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