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Quinacrine Stimulation of Glutathione Reduction Dependent on the Presence of a Particulate Brain Subfraction[1] (38462)

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One of the functions of the hexose monophosphate (HMP) shunt is to provide reduced NADP for the reduction of oxidized glutathione (GSSG). Nervous tissue contains the enzymes of the HMP shunt pathway, NADPH-specific glutathione reductase (1, 2), and relatively high concentrations of reduced glutathione (GSH) (3). Since it has been suggested that a function of glutathione in cells may be that of maintaining sulphydryl groups, essential for enzyme activity and membrane integrity, in the reduced state, we have been interested in studying the HMP shunt-dependent GSSG reduction in nervous tissue and the effects of some CNS-active agents on these reactions. Studies with guinea pig brain preparations showed that both the HMP shunt pathway and GSSG reduction were stimulated by chlorpromazine [2-chloro-10-(3-dimethylaminopropyl)-phenothiazine], and that this effect required the presence of a particulate subcellular fraction (4). During these investigations, we found that other compounds with known CNS effects were able to affect this system; among these was quinacrine [3-chloro-7-methoxy- 9-(1- methyl- 4- diethylaminobutylamino) acridine dihydrochloride], one of a family of drugs which precipitate hemolysis in individuals with erythrocyte HMP shunt enzyme deficiencies. We found that quinacrine was effective in stimulating HMP-dependent GSSG reduction in brain preparations and that the effect was dependent on the presence of a particulate subcellular fraction of brain.

Materials and Methods. The cerebral hemispheres were removed from normal male guinea pigs (300–500 g) and immediately disrupted in 9 vol of cold 0.25 *M* sucrose in a Potter–Elvehjem homogenizer. Subcellular fractions were then prepared by centrifuging, in each case, for 20 min at 4° in a refrigerated Servall RC-2. Supernatant fractions are identified in the text by the letter S, preceded by a number, in 1000*g* units, representing the gravitational force at which they were prepared (*e.g.*, 12 S denotes the supernatant fraction obtained by centrifuging at 12,000*g* for 20 min). Pellet fractions are designated by the letter P, preceded by two numbers; the first figure designates the centrifugal force used to prepare the supernatant fraction that was then subjected to the second gravitational force for the sedimentation of the particulate fraction (*e.g.*, 12-20 P refers to the pellet obtained by centrifuging the 12 S at 20,000*g* for 20 min). Pellets were washed once by suspending in 0.25 *M* sucrose. The reprecipitated pellets were finally resuspended in the same medium, commonly to one-half the volume from which they were isolated, and used in the experiments.

Glutathione reduction was measured essentially as described previously (4). Final concentrations of assay components in each flask, which also contained 0.1 ml of the appropriate supernatant fraction, were 50 m*M* imidazole, pH 7.0, or in some experiments pH 7.2, 2.5 m*M* glucose-6-phosphate (Sigma Chemical Company), 0.5 m*M* NADP (Sigma), 2.5 m*M* GSSG (Sigma), and 100 m*M* KCl; quinacrine (Nutritional Biochemicals) was added as noted in the text. When a particulate fraction was used, it was added in the amount required to restore the ratio of supernate to particulate material found in the original homogenate. Sulphydryl (SH) contents were determined using Ellman's reagent, 5,5′-dithiobisnitrobenzoic acid (DTNB; Aldrich Chemical Company) (5). Results are expressed as μmoles SH formed per hour per gram wet

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TABLE I. Effects of Chlorpromazine and Quinacrine on Glutathione Reduction.

Brain preparation	Drug addition	SH formed (μmoles/hr/g)[a]	% Control
1 S	None	161.1 ± 4.9	—
	0.5 m*M* Quinacrine	232.4 ± 6.4	144 ± 5
12 S	None	167.0 ± 4.3	—
	0.5 m*M* Quinacrine	231.4 ± 5.6	139 ± 4
	2.0 m*M* CPZ	239.3 ± 6.1	143 ± 3
37 S	None	186.8 ± 3.9	—
	0.5 m*M* Quinacrine	192.4 ± 4.7	103 ± 3
	2.0 m*M* CPZ	183.7 ± 6.4	98 ± 4

[a] Results expressed as averages ± SEM.

weight of tissue.

Results. As previously reported (4), the addition of CPZ to guinea pig brain preparations resulted in a stimulation of GSSG reduction by the hexose monophosphate shunt, and this effect was retained in the supernatant fraction obtained after centrifuging at 12,000*g* (Table I). Similarly, quinacrine addition to these brain preparations resulted in increased SH formation. However, the ability of quinacrine to stimulate GSSG reduction disappeared when the 37 S fraction was used for assay. The results illustrate the comparable response of GSSG reduction to CPZ and quinacrine in the 12 S fraction and the disappearance of the stimulatory effects of the drugs upon removal of material sedimented by centrifuging at 37,000*g*. The dependence of the stimulatory effect of quinacrine upon drug concentration was examined in preparations of the 12 S fraction; the results obtained as increasing concentrations of quinacrine were added to this fraction are given in Table II. Increasing response to the drug is noted to a maximum reached at 1–2 m*M* quinacrine.

In the case of CPZ it was reported that the addition of the sedimented material back to the 37 S fraction restored the effectiveness of this drug; further, differential centrifugation studies showed that most of the stimulatory response to CPZ was absent from the 20,000*g* supernatant fraction (4). Similar studies using quinacrine have revealed that most of the ability to restore the stimulatory effect of this latter agent is associated with the 12-20 P (Table III), while the 20-37 P displays only a slight ability to restore the effectiveness of quinacrine in the 37 S fraction. That the stimulation is dependent on the presence of the 12-20 P fraction was further demonstrated by experiments which showed that adding less of this preparation to the incubation medium resulted in a correspondingly decreased effect of quinacrine (unreported observations).

Additional studies have shown that doubling the concentration of substrates or cofactor did not affect the results; further, the effects of quinacrine were maintained when nicotinamide, an NADP'ase inhibitor, was included in the assay medium. The course of the reaction, using the 12

TABLE II. Effect of Varying Quinacrine Concentrations.[a]

Final concentration of quinacrine (m*M*)	SH formed (μmoles/hr/g)[b]	% Control
0.0	185.5 ± 0.7	—
0.1	209.2 ± 1.8	113 ± 2
0.5	246.5 ± 3.6	133 ± 3
1.0	257.8 ± 2.4	139 ± 2
2.0	262.1 ± 2.8	141 ± 3
4.0	263.4 ± 12.8	142 ± 5

[a] Incubations carried out in imidazole buffer, pH 7.2, using 12 S fraction.
[b] Results expressed as averages ± SEM.

TABLE III. Effects of Addition of Particulate Fractions to 37 S.

Fraction added	SH formed (μmoles/hr/g)[a]		
	Control	+0.5 m*M* Quinacrine	% Control
—	181.9 ± 8.1	185.0 ± 9.7	102 ± 2
12 - 20 P	156.3 ± 7.6	206.5 ± 8.2	132 ± 3
20 - 37 P	164.9 ± 11.4	187.7 ± 10.7	114 ± 5

[a] Results expressed as averages ± SEM.

S fraction, in the presence and absence of 10 m*M* nicotinamide is shown in Fig. 1; when nicotinamide is present, all activities are increased slightly, but the effect of quinacrine remains evident. The reaction is linear with time in the presence or absence of quinacrine through 30 min under the assay conditions described.

Discussion. These studies show that quinacrine, an antimalarial drug with known CNS effects, stimulates the reduction of GSSG in preparations of guinea pig cerebral hemispheres. This stimulatory effect of quinacrine was only manifested in the presence of a particulate, subcellular fraction, similarly to those reported for chlorpromazine (4). Other studies in our laboratories (unpublished results) have shown that quinacine, like CPZ, stimulated the HMP shunt activity (measured by the oxidation of ^{14}C-1-G6P) and the reduction of GSSG in a parallel manner. This would imply that the effect of quinacrine on GSSG reduction is not due to a drug-metabolizing system that may be utilizing or producing NADP or NADPH. It further appears that the stimulation of GSSG reduction by quinacrine does not involve the preservation of substrates or cofactor, since increasing the amounts of these compounds in the assay medium neither enhanced the rate of the reaction in the presence or absence of quinacrine, nor diminished the response to drug addition. Although the addition of nicotinamide increased the reduction of GSSG slightly, nicotinamide did not abolish the stimulatory effects of quinacrine, indicating that the stimulation of GSSG reduction by quinacrine was not due to a NADP-preserving effect of the drug.

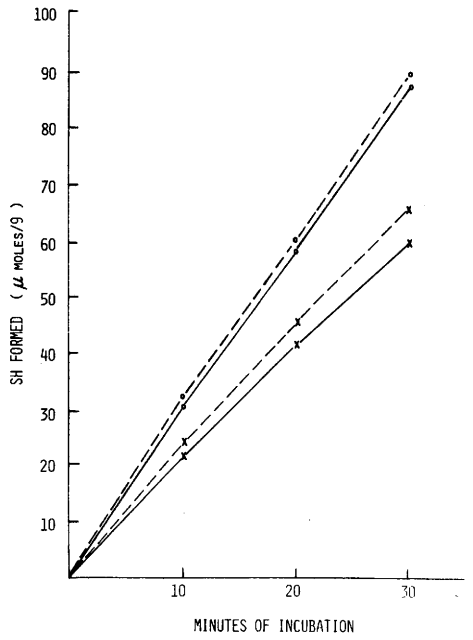

FIG. 1. The stimulation of GSSG reduction in the 12 S fraction by quinacrine in the presence or absence of nicotinamide. The 12 S fraction (×) and the 12 S fraction plus 1 m*M* quinacrine (o) were assayed in the absence (solid lines) and presence (dashed lines) of 10 m*M* nicotinamide. Incubations were carried out in imidazole buffer, pH 7.2.

If the HMP shunt is operating in brain in part to provide NADPH for GSSG reduction and the maintenance of essential SH groups in the reduced state (6), the effects of CNS-active agents on this system may be of physiological significance, especially in view of the suggestion that the state of oxidation of certain sulphydryl groups in the membranes of cells of the nervous system may play an important role in the transmission of nervous impulses (7). The implications of our findings might be questioned because the concentrations of the drugs used in these studies are considerably higher than plasma levels found in man after administration of therapeutically effective doses (8). However, the effects may be significant in light of animal studies that have shown that CPZ was concentrated in brain tissue (9, 10) and that this drug was tightly bound to brain proteins (11).

The requirement for the particulate cellular material in order for these drugs to exert their stimulatory effect is currently under investigation. In view of the reports that CPZ binds to proteins and cellular membranes (11) and the suggestions that this drug can affect the conformation of membrane components (12), the possibility exists that the effective material is a component of nerve cell membranes which is acting as a mediator of the drug effects on the soluble enzymes responsible for the reduction of glutathione. The determination of the subcellular localization of the active fraction is thus of interest, and this problem is currently being studied. In addition, attempts are in progress to obtain a soluble preparation from the subcellular particles to be used in the isolation and characterization of the material whose presence is required in order for quinacrine to exert its stimulatory effects on the reduction of glutathione in brain.

Summary. Glutathione reduction by the hexose monophosphate shunt was shown to be stimulated by quinacrine in preparations of guinea pig cerebral hemispheres. The stimulatory effect of the drug depended on the presence of a subcellular, particulate fraction obtained by differential centrifugation.

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