Effect of Convulsant and Anticonvulsant Agents on the Activity of Cytochrome Oxidase.*† (18034)

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The cerebral oxygen consumption is known to be increased during convulsive seizures. Hence, an attempt was made to ascertain whether chemical agents known to induce convulsive seizures modify the activity of enzymes involved in oxygen consumption. The present report is a summary of the effects of convulsant and anticonvulsant agents on the activity of cytochrome oxidase.

Method. The effect of convulsant and anticonvulsant agents on the activity of cytochrome oxidase was investigated following the method of Schneider and Potter(1) as described by Umbreit and collaborators(2) consisting of measuring the oxygen uptake by an enzyme-substrate mixture. 0.3 ml of a $1x10^{-3}$M $AlCl_3$ solution, 0.3 ml of either water or a solution containing the convulsant and anticonvulsant agents in concentrations from $1x10^{-2}$ to $1x10^{-4}$M, either 0.5, or 0.75, or 1 ml of a suspension containing 0.2% tissue homogenate in water, and water in amounts to bring the total volume to 1.6 ml were added to the main chamber of a Warburg vessel. 0.1 ml of a 0.2 N sodium hydroxide solution with 1 sq cm folded filter paper was placed in the center cup. One ml of a 0.1M phosphate buffer at pH 7.4 containing cytochrome c in a concentration of $2.4x10^{-4}$M was added to one side arm of the Warburg vessel and 0.3 ml of a 0.114M sodium ascorbate solution at pH 7.4 was added to the other side arm. All water used was twice distilled in glass to avoid contamination with heavy metals. The solutions were ice-cold and were freshly prepared. During preparation the Warburg vessels were immersed in ice. Air was used as the gas phase for convenience. The water bath was kept at 37°C. After thermal equilibrium was reached the ascorbate and the cytochrome c were tipped from the side arms into the main chamber and the rate of oxygen uptake was measured. Readings were made every 10 minutes for half an hour. The oxygen uptake was approximately proportional to the time of incubation. The oxygen uptake in parallel runs differed less than 3%.

Preparation of the enzyme. Either rat liver or rat brain served as a source of the enzyme. Immediately before mounting the Warburg vessels containing the substrate mixture the animals were decapitated and a tissue aliquot was measured. The aliquot was homogenized by means of an apparatus described by Potter and Elvehjem(2,3) under ice, it was diluted with distilled water, and was added to the main chamber of the Warburg vessel. The endogenous oxygen uptake of the liver was insignificant.

Convulsant and anticonvulsant agents. The substances were used in low concentrations ($1x10^{-3}$M and less) at pH 7.4. This precaution was necessary to minimize changes in the buffering ability of the phosphate solution, a factor that modifies the activity of cytochrome oxidase by itself. Furthermore, many of the substances induced changes in the rate of autoxidation of the ascorbate if

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1. Schneider, W. C., and Potter, V. R., *J. Biol. Chem.*, 1943, v149, 217.

2. Umbreit, W. W., Burris, R. H., and Stauffer, J. F., *Manometric Techniques and Related Methods for the Study of Tissue Metabolism*, Burgess Publishing Co., Minneapolis, Minn., 1945.

3. Potter, V. R., and Elvehjem, C. A., *J. Biol. Chem.*, 1936, v114, 495.

used in final concentrations of $1x10^{-2}$M and more.

Autoxidation of the substrate. The rate of the autoxidation of the ascorbate was determined by extrapolation to zero tissue concentration from a series of 3 different tissue concentrations respectively(2). The autoxidation rate was obtained by subtracting the increments of oxygen uptake in the Warburg vessels containing 0.75 ml and 1 ml of homogenate from the oxygen uptake in the Warburg vessels containing 0.5 ml of the homogenate. The rate of oxygen uptake in 10 minutes averaged 5 μl. A similar oxygen uptake of 5 μl per 10 minutes was found in incubated control mixtures containing the above enumerated mixture without homogenate, and the above enumerated mixture containing tissue homogenate and cyanide in amounts giving a final concentration of $1x10^{-2}$M.

The rate of autoxidation of ascorbate in the presence of the convulsant and anticonvulsant agents was determined: (1) by extrapolation to zero tissue concentration from a series of 3 different tissue concentrations for each agent in concentrations of $1x10^{-3}$ to $1x10^{-5}$M; (2) by measuring the oxygen uptake of mixtures containing the agents, the substrate-mixture in the absence of tissue homogenate; and (3) by measuring the oxygen uptake of mixtures containing the agents, the substrate-enzyme mixture, and cyanide in amounts to give a final concentration of $1x10^{-2}$M. The oxygen uptake in 10 minutes averaged in most instances 5μl.

Calculation. The oxygen uptake due to autoxidation of the substrate was subtracted from the oxygen uptake of the substrate-enzyme (1 ml) mixture. The 3 point extrapolation to zero tissue concentration yielded straight lines and the intercept for the autoxidation rates was in almost all instances approximately 5 μl. The oxygen uptake of controls without convulsant and anticonvulsant agents was taken as 100%, and the oxygen uptake of mixtures containing the convulsant and anticonvulsant agents was expressed as a percentage of it. The effect of the agents on the activity of cytochrome oxidase was considered significant when the oxygen uptake deviated from 100% by more than twice the square root of the sum of the standard error of the mean of controls (mixtures without convulsant and anticonvulsant agents) and the standard error of the mean of a group of experiments (mixtures containing one concentration of one agent) $[2\sqrt{\text{S.E.}^2(\text{control}) \pm \text{S.E.}^2(\text{exp.})}]$.

Results. The results are summarized in Table I. The activity of cytochrome oxidase increased in the presence of the convulsant agents used (acetylcholine, caffeine, camphor, dichlorodiphenyltrichloroethane, methylsalicylate, pentamethylenetetrazol, picrotoxin, scilliroside, and strychnine) in concentrations of $1x10^{-3}$M and less. The greatest increase observed was over 150%. The activity of cytochrome oxidase was not modified significantly by the anticonvulsant agents used (hydantoin, methyl-phenyl-ethyl hydantoin, phenylhydantoinate sodium, phenobarbital, and tridione) in concentrations of $1x10^{-3}$M and less. The oxygen uptake in the presence of enzyme from various sources was different, but the effect of convulsant and anticonvulsant agents on the activity of cytochrome oxidase was approximately the same in the complete series using liver homogenate and in the few representative experiments using brain homogenate as the source of the enzyme.

Discussion. The observation that anticonvulsant agents fail to modify significantly the activity of cytochrome oxidase is in agreement with the observation that nembutal and similar agents depress neurones by some mechanism that is independent of azide and cyanide sensitive oxidative enzyme systems, *e.g.* the cytochromes(4).

The increased activity of cytochrome oxidase in the presence of the convulsant agents suggests that at some phase of their action the convulsant agents actively increase the cerebral oxidative processes. Theoretically it is possible that the well known increase of oxygen consumption of the brain during convulsive seizures is implicated as follows: (1) as a factor initiating seizures; and (2) as a part of the mechanisms through which each unit recovers after every discharge. Since

4. Larrabee, M. G., Ramos, J. G., and Bulbring, E., *Fed. Proc.*, 1950, v9, 75.

TABLE I.
Effects of Agents on Activity of Cytochrome Oxidase (Rat Liver).

Agents	Oxygen uptake in mixtures containing the agents expressed in % of the oxygen uptake of the control* Concentration of the agents in mols: 1×10^{-3}	1×10^{-4}	1×10^{-5}
Acetylcholine	122†	140	160
Caffeine	163	152	127
Camphor	192	133	107
Dichlorodiphenyltrichloroethane	216	131	120
Methylsalicylate	183	131	124
Pentamethylene tetrazol	181	160	142
Picrotoxin	176	171	129
Scilliroside	238	202	189
Strychnine	139	173	139
Hydantoin	124	103	100
Methyl-phenyl-ethyl hydantoin	108	108	104
Phenobarbital	111	102	98
Phenylhydantoinate sodium	—	110	101
Tridione	120	102	97
Choline	193	96	98

* The standard error of the mean for each value was less than ±5%. Each value represents the average of 10 separate experiments.

† The ascorbate Q_{O_2} (dry liver after correction for incomplete homogenization) averaged 305. This value represents the 100%.

changes in the electrical activity of brain characteristic for convulsive seizures precede the increase of cerebral oxygen consumption (5), it is likely that the increase of oxygen consumption is not a precipitating factor of convulsive seizures.

The above presented results indicate, therefore, that convulsant agents activate at least 2 processes: (1) a non-oxidative mechanism that initiates the seizures; and (2) the oxidative recovery processes.

5. Davies, P. W., and Remone, A., *Research Publications of the Assn. for Research in Nervous and Mental Disease*, 1947, v26, 205.

Summary. 1. The activity of cytochrome oxidase increased in the presence of convulsant agents used in concentrations of $1x10^{-3}$M and less. 2. The activity of cytochrome oxidase was not significantly modified by the anticonvulsant agents used in concentrations of $1x10^{-3}$M and less. 3. Convulsant agents, therefore, activate at least 2 processes, namely, a non-oxidative mechanism that initiates the seizures and the oxidative recovery processes occurring in each unit during the convulsive seizures.

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