SPASMOLYTIC EFFECT OF THE PAPAVERINE AND INHIBITION OF THE OXIDATIVE PHOSPHORYLATION

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In the course of investigations conducted during the last years, we have found, with isolated guinea pig ileum, that papaverine chiefly inhibits the "tonic phase" of the acetylcholine, histamine, $BaCl_2$-induced contraction; in contrast the "spike phase" is unaffected by the drug used within a large concentration range.

It is generally agreed that similar effects are elicited by chemical (KCN) or physical anoxia and by 2,4 dinitrophenol (2,4DNP), at proper concentrations (Schmitt and Nicoll, 1933; West et al. 1951; Furchgott and Shorr 1950; Starkenstein 1941). Thus, the normal aerobic response of the intestinal smooth muscle "may be considered a biphasic response; anoxia abolishes the tonic phase, but the spike phase persists" (West et al. 1951). 2,4 DNP elicits the same effects by impairing the synthesis of high energy phosphate bonds (West et al. 1951).

These findings open the question whether the miolytic action of papaverine might be ascribed to some interference with energy production, reflected in the inability of the smooth muscle to maintain tone.

To examine this hypothesis we have studied the papaverine action on the oxidative phosphorylation in rat liver mitochondria, prepared according to Hogeboom (1955). The O_2 uptake was measured manometrically at 26°C in the Warburg

apparatus; inorganic P (P_i) was determined according to Fiske and Subbarow (1925); protein by the biuret method (Gornall et al 1949). The data (Table 1) can be so summarized in the following terms :

a) papaverine strongly inhibits the O_2 uptake of rat liver mitochondria oxidizing glutamate and β-hydroxybutirate under phosphorylative conditions;

b) phosphate uptake decreases in a parallel fashion and the P/O ratio remain unchanged.

Table 1. Effect of the papaverine on oxidative phosphorylation

Substrate	Papaverine mM	Oxigen uptake		P_i uptake	
		µatoms	inhib.%	µmoles	inhib.%
L-Glutamate	-	4.70	-	12.1	-
"	0.05	0.70	85.0	0.8	94.0
"	0.01	1.24	73.5	3.2	73.0
"	0.005	2.30	51.0	6.4	47.0
"	0.001	3.30	30.0	9.7	20.0
D-L- β-Hydroxybutyrate	-	2.94	-	6.45	-
"	0.05	0.62	78.8	0.80	87.6
Succinate	-	7.40	-	14.5	-
"	0.05	7.20	3.0	10.0	31.0
L-Glutamate+2,4-DNP	-	6.50	-	-	-
" "	0.05	0.35	94.5	-	-
" "	0.01	1.34	78.0	-	-
" "	0.001	3.20	51.0	-	-

Each Warburg vessel contained : 15 mM KH_2PO_4 buffer pH 7.5, 30 mM Tris buffer pH 7.5, 1 mM EDTA pH 7.5, 5 mM $MgSO_4$, 10 mM substrate (20 mM β-hydroxybutyrate), 30 mM glucose, 0.5 mg yeast hexokinase (Sigma, Type III), 1.3 mM ATP, 90 mM sucrose, 4-5 mg of protein rat-liver mitochondria. With 0.1 mM 2.4 DNP, glucose and hexokinase were omitted. Final volume 2 ml; 0.2 ml 1.8 M KOH and filter paper in the centre well; time of incubation 20 min, gas phase air, temperature 26°.

c) with succinate as substrate, O_2 uptake is unaffected by papaverine, P_i uptake is slightly decreased presumably by the inhibition of phosphorylation due to the oxidation of DPN-substrates;

d) 2,4 DNP is unable to abolish the effects of papaverine.

These data suggest that papaverine inhibits the electron transfer reactions in the respiratory chain between DPN and flavoproteins. Such a statement is mainly supported by the failure of papaverine to inhibit succinate oxidation and by its activity in a~P acceptors-deficient system activated by 2,4 DNP. In this respect, the mechanism of action of papaverine is similar to that of amytal (Ernster et al. 1955), allyloxybenzamide (Bruni and Contessa 1961), and rotenone (Ernster et al. 1962); the degree of activity is very high and greater than that of amytal, so that papaverine can be routinely employed as the inhibitor of choice in research on oxidative phosphorylation.

Concerning the pharmacological implications of our findings, we emphasize that papaverine affects the acetylcholine, histamine, $BaCl_2$-responses of intestinal smooth muscle in a similar manner than 2,4 DNP and chemical (KCN) or physical (O_2-lack) anoxia, by suppressing the "tonic phase" of the contraction, but not the "spike phase". The data so far obtained suggest that the inhibition of the respiratory chain by papaverine may play an important part in the spasmolytic effect of the drug, by impairing the synthesis of high energy phosphate bonds. An alternative hypothesis will be discussed in the paper "in extenso" (Santi in press).

The main papaverine derivatives and other spasmolytic agents are under investigation.

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