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ON THE MECHANISM OF SPASMOLYTIC EFFECT OF PAPAVERINE AND CERTAIN DERIVATIVES*†

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Abstract—The authors have compared the spasmolytic activity and the effect on oxidative phosphorylation of papaverine and some of its derivatives (dihydropapaverine, ethaverine and eupaverin).

With guinea pig ileum and rabbit duodenum, papaverine, dihydropapaverine and ethaverine faithfully mimic the effect of anoxia, cyanide, 2,4-DNP or other enzyme inhibitors, by suppressing the "tonic phase", of acetylcholine, histamine, $BaCl_2$-induced contraction, without affecting the "spike phase" according to West *et al.*[1]

Papaverine, dihydropapaverine, ethaverine strongly inhibit the oxygen uptake of rat liver mitochondria oxidizing glutamate under phosphorylative conditions. This effect is not reversed by 2,4-DNP. With succinate as substrate the oxygen uptake is unaffected by these drugs. The results suggest that the inhibition takes place in the electron-transfer reactions chain between nicotinamide-adenine dinucleotide and cytochrome b. Papaverine and ethaverine show the greatest activity both in experiments with isolated gut and with rat liver mitochondria. Eupaverin demonstrated a peculiar behaviour, because it failed to give an "anoxia-like" effect on the isolated gut and its mechanism of action on rat liver mitochondria is rather different. Possible relations between these biochemical effects of the drugs and their spasmolytic activity are discussed.

THE response of intestinal smooth muscle to several stimulant drugs is similarly affected by anoxia, cyanide and 2,4-DNP.[1] Under these experimental conditions, the immediate rapid contraction remains unaffected, whereas the outcoming state of increased tonus is abolished. Thus, the two normal components of the intestinal smooth muscle contraction (the "spike phase" and the "tonic phase", according to West *et al.*[1]) differ remarkably from each other, the latter appearing strictly aerobic because it is abolished by anoxia and cyanide or by impairing (by addition of 2,4-DNP) the synthesis of ATP, normally coupled to the electron-transport in the respiratory chain.

Other enzyme inhibitors (sodium fluoroacetate, propionate and malonate) are reported to affect the responses of the intestinal smooth muscle in a similar manner.[1]

With regard to the pharmacological implications, it seems noteworthy that Pv and some of its derivatives selectively inhibit the "tonic phase" of the acetylcholine,

* *The abbreviations used are as follows*: Pv = papaverine (6,7-dimethoxy-1 (3′4′-dimethoxybenzyl) isoquinoline; Eth = ethaverine (perparin) (6,7-diethoxy-1(3′4′-diethoxybenzyl) isoquinoline; DPv = dihydropapaverine (6,7-dimethoxy-3,4-dihydro-1 (3′4′-dimethoxybenzyl) isoquinoline; EPv = eupaverin (6,7-dimethoxy-3-ethyl-1 benzyl-isoquinoline) 2,4 DNP = 2,4-dinitrophenol; NAD = nicotinamide-adenine dinucleotide (DPN). Eupaverin and dihydropapaverine were kindly supplied by Bracco Industria Chimica; ethaverine by Biologici Italia S.p.A.

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histamine, $BaCl_2$-induced contraction; the "spike phase", on the contrary, is unaffected by these spasmolytic drugs within a large concentration range[2, 3] (Fig. 1). These findings open the question whether the mechanism of action of certain spasmolytic agents might be ascribed to interference with energy production, reflected in the inability of the smooth muscle to maintain a prolonged state of increased tonus. In order to examine this hypothesis, we have comparatively studied the spasmolytic activity and the effect on the oxidative phosphorylation of Pv and its main derivatives.

In a previous paper, Coker and Mija[4] reported that Pv inhibits the oxygen uptake of slices of rat duodenum and, surprisingly, it was inactive on homogenate.

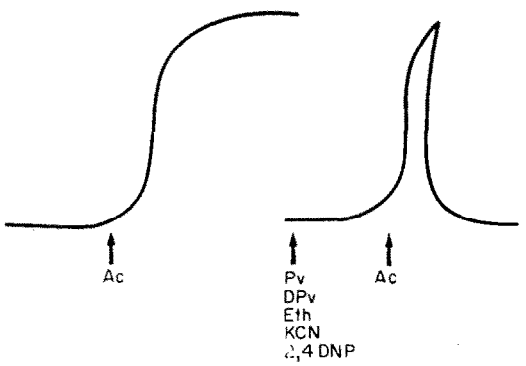

FIG. 1. Effect of spasmolytic drugs (papaverine, dihydropapaverine, ethaverine) and enzyme inhibitors (KCN and 2,4 DNP) on the response of isolated guinea pig ileum to acetylcholine.

MATERIALS AND METHODS

The spasmolytic activity of Pv, DPv, Eth and EPv was tested on guinea pig ileum and rabbit duodenum suspended in a 30 ml bath containing Tyrode medium at 37°C, in which air was bubbled through the bath fluid. For each drug was determined the lowest concentration which was able to abolish the "tonic phase" of acetylcholine, histamine, $BaCl_2$-induced contraction without affecting the "spike phase". A 20-min dose cycle was used, the spasmolytic drugs being allowed to act for 2–3 min. In order to study the effect of anoxia, air bubbling through the bath fluids was replaced with a nitrogen-CO_2 mixture (95% nitrogen and 5% CO_2) 20 min before the addition of acetylcholine. Further experiments were performed by adding KCN at final concentrations of 1–2 μg/ml, according to West *et al.*[1]

Concentrations of 2,4-DNP ranging from 20 to 40 μg/ml were ascertained to be the most suitable to obtain effects comparable with those elicited by anoxia.

The action of Pv, Eth, DPv and EPv on the oxidative phosphorylation was tested by using rat liver mitochondria, prepared according to Hogeboom;[5] the final suspension was made in 0·25 M sucrose with a concentration of 7 mg of protein/ml. The oxygen uptake was followed manometrically at 26°C in the Warburg apparatus; the medium composition is indicated in the legend of Table 1. Inorganic P(Pi) was determined by procedure of Fiske and Subbarow[6] and protein was determined by the biuret method.[7]

RESULTS

Effect on the response of intestinal smooth muscle to acetylcholine and other stimulant drugs

In agreement with the previous findings, using guinea pig ileum and rabbit duodenum, oxygen lack, KCN and 2,4-DNP were found to give selective inhibition of the "tonic phase" of the acetylcholine, histamine, $BaCl_2$-induced contraction, without

TABLE 1. EFFECT OF PAPAVERINE, ETHAVERINE, DIHYDROPAPAVERINE AND EUPAVERIN ON OXIDATIVE PHOSPHORYLATION.

Substrate	Drugs (mM)	Oxygen uptake		Pi uptake	
		(μatoms)	(inhib. %)	(μmoles)	(inhib. %)
L-Glutamate	—	7·4	—	23·4	—
,,	Pv 0·05	0·5	93·2	1·6	93·1
,,	Eth 0·05	0·2	97·2	1·6	93·1
,,	DPv 0·10	2·0	72·9	7·2	69·2
	EPv 0·10	5·4	27·0	14·5	38·0
Succinate	—	5·7	—	14·5	—
,,	Pv 0·05	5·3	7·0	11·3	22·0
,,	Eth 0·05	4·9	14·0	8·9	38·6
,,	DPv 0·10	6·0	0·0	12·1	16·5
,,	EPv 0·10	4·9	14·0	9·6	33·7

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, 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. 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°.

affecting the "spike phase". Among the spasmolytic drugs employed, this effect was elicited by Pv, Eth and DPv. In this respect the degree of activity was found to be of the same order for Pv and Eth, and four to eight times less for DPv. In experiments conducted with guinea pig ileum, the most suitable concentrations ranged from 0·0055 mM to 0·020 mM for Pv and Eth and about 0·030 mM for DPv, whereas, experiments with rabbit duodenum required greater concentrations (about 0·030 mM for Pv and Eth, 0·055–0·13 mM for DPv). Under the same experimental conditions, EPv failed to give a selective inhibition of the "tonic phase" because its spasmolytic activity also remarkably lowered the "spike phase". The full inhibition of the "tonic phase", however, occurred at the same concentrations required by DPv.

Effect on oxidative phosphorylation

Respiration stimulated by P-acceptor. Table 1 shows the effect of Pv, Eth, DPv and EPv on rat liver mitochondria oxidizing glutamate or succinate under phosphorylative conditions.

The oxidation of glutamate is depressed by the drugs tested with a simultaneous decrease of Pi uptake. The inhibition of phosphorylation closely parallels that of respiration; thus the P/O ratio remains unchanged. 0·05 mM Pv and Eth—the most active compounds—determine an almost complete inhibition of oxygen and phosphate uptake. About 20–30 per cent inhibition is still present at 0·0005 mM (Fig. 2).

EPv is a weakly active derivative: 0·1 mM produces an effect comparable to that determined by 0·01 mM DPv and 0·001 mM Pv or Eth (Fig. 2).

With succinate as substrate (Table 1) the activity of Pv and Eth on oxygen uptake appears very weak when compared with the strong inhibition of the oxidation of glutamate. 0·1 mM DPv shows a complete inactivity on oxygen consumption whereas EPv, unlike the other compounds, determines an inhibition of the oxidation of succinate which approximates that produced on the oxidation of glutamate. Pi uptake—with succinate as substrate—exhibits a constant depression which exceeds that of oxygen uptake (Table 1); this effect could be ascribed to the inhibition, by the drugs tested, of that part of phosphorylation coupled to the oxidation of NAD-linked substrates originating by succinate.

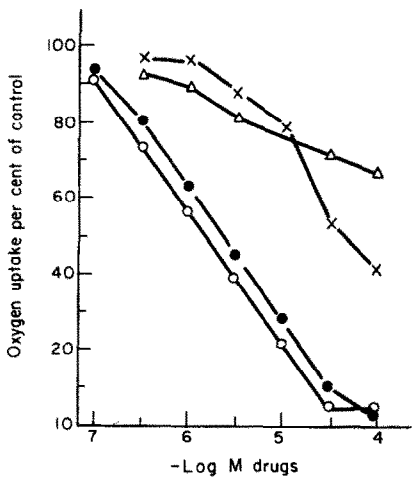

FIG. 2. Effect of Pv (●—●) Eth (○—○), DPv (×—×) and EPv (△—△) at various concentrations on mitochondrial respiration stimulated by P-acceptor. Experimental conditions as in Table 1; substrate glutamate.

With regard to Pv and Eth the strong inhibition of the oxidation of glutamate together with the weak activity on the oxidation of succinate, suggests a site of action located between NAD and cytochrome b. DPv shows a similar mechanism of action, but more selective since it is lacking activity on the oxidation of succinate. By a comparison between the activity displayed by EPv with glutamate and with succinate as substrates, it appears that—unlike the other compounds—an inhibition of the cytochrome system takes an important part in the effect of this drug on oxidative phosphorylation.

Respiration stimulated by 2,4-*DNP*. In order to elucidate whether the energy-transfer reactions are involved in the action of Pv-like drugs a comparison was made between their activity in a phosphorylating and a non-phosphorylating system. Thus experiments were carried out on mitochondria oxidizing glutamate in the presence of 2,4-DNP at uncoupling concentration. The data so far obtained (Fig. 2 and 3)

indicate that the inhibition of the respiration by the drugs tested is unaffected by 2,4-DNP. This fact demonstrates that the action of Pv-like drugs is independent of the presence of the phosphorylative processes and develops in a direct manner on the electron-transfer reactions.

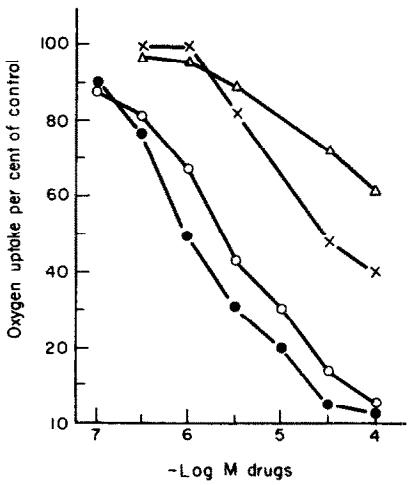

FIG. 3. Effect of Pv (●—●), Eth (○—○), DPv (×—×) and EPv (△—△) at various concentrations, on mitochondrial respiration stimulated by 0,1 mM 2,4-DNP. Experimental conditions as in Table 1; substrate glutamate; glucose and hexokinase were omitted.

DISCUSSION

The findings reported in this paper indicate that Pv and some other compounds structurally related (Eth, DPv) strongly inhibit the electron-transfer reactions in the respiratory chain. The data supporting this statement, important for the localization of the site of action, can be summarized in the following terms: (a) with NAD-linked substrates oxygen uptake is inhibited and phosphate uptake decreases in a parallel fashion; thus the P/O ratio remains unchanged; (b) with succinate as substrate, oxygen uptake is unaffected and phosphate uptake is slightly diminished, presumably by inhibition—by the drugs tested—of the phosphorylation coupled to the oxidation of NAD-linked substrates originated by succinate; (c) 2,4-DNP is unable to remove the above mentioned effects. The oxidation of succinate thus remains unaffected but the oxidation of NAD-linked substrates is depressed and this effect can be detected even in a ~ P acceptor-deficient system, which is activated by 2,4-DNP. It appears that the inhibition by Pv, Eth and DPv, takes place between NAD and cytochrome b; the site of action is therefore the same as the one recognized for amytal,[8] rotenone[9] and allyloxibenzamide.[10–12]

The activity of Pv and Eth is so great that these spasmolytic agents can be routinely employed as inhibitors of choice in research on oxidative phosphorylation, thus replacing the weakly active amytal.

Evidence suggests that Pv and some analogues (Eth, DPv) faithfully mimic the effect of anoxia, cyanide, 2,4-DNP or other enzyme inhibitors, by suppressing the "tonic phase" but not the "spike phase" of smooth muscle contraction. This behaviour

is in agreement with the above mentioned biochemical effects and suggests that the impaired synthesis of high-energy phosphate bonds, produced by the inhibition of the respiratory chain, might play an important role in the mechanism of action of some spasmolytic agents.

An inconsistency has been however ascertained, since EPv, despite its spasmolytic activity, exercises very little inhibition of oxidative phosphorylation; on the other hand—as it has been mentioned—EPv failed to determine an "anoxia-like" effect on the isolated gut, and its mechanism of action may deserve further investigation. With regard to the structure-activity relationship, this fact prompts the opportunity of examining other Pv derivatives as well as the convenience of studying the effect of these drugs on other metabolic steps involved with energy production.

Further research will be designed to develop these points and to investigate whether other pharmacological properties of Pv-like drugs, besides the spasmolytic effect, may be related to this inhibition of oxidative phosphorylation.

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