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Effects of papaverine and eupaverin on calcium uptake by isolated sarcoplasmic vesicles

F. CARPENEDO, G. C. TOSON, M. FURLANUT AND M. FERRARI

Institute of Pharmacology, University of Padua, Padua, Italy

Papaverine and eupaverin increase the rate of uptake of calcium by sarcoplasmic vesicles isolated from rabbit white skeletal muscle. The degree of activity of the above drugs is clearly affected by changes of ATP, oxalate and Ca^{2+} concentrations. The results are discussed in view of present knowledge about the effects of papaverine-like drugs upon muscular contraction.

The effects of various spasmolytic agents have been reliably referred to an interference with the essential role of calcium ions in muscular function (Daniel, 1964; Ferrari, 1964; Ferrari & Carpenedo, 1965; Tóth, Ferrari & others, 1966; Ferrari, 1970). These views presumably apply not only to the activity of spasmolytics on smooth muscle, but also to the effects of these drugs on skeletal muscle, where papaverine and eupaverin exert contracture or inhibition, depending on the concentrations (Buttar, 1969; Carpenedo, unpublished observation).

However, present knowledge about the influence of papaverine-like drugs on calcium ion movements is unsatisfactory. Only indirect conclusions have been made, suggesting an interference by spasmolytic agents with Ca^{2+} influx or binding at some cellular components, or both (Imai & Takeda, 1967; Ferrari & Carpenedo, 1968; Tashiro & Tomita, 1970).

We have now examined the effects of papaverine and eupaverin on Ca^{2+} uptake by sarcoplasmic reticulum, a model which has been largely employed to study the effects of drugs on Ca^{2+} movements at membrane level (Martonosi & Feretos, 1964; Inesi, Goodman & Watanabe, 1967; Balzer, Makinose & Hasselbach, 1968).

METHODS

All experiments were made with a 10 000–50 000 *g* centrifugal fraction of rabbit white skeletal muscle precipitated in 10% sucrose-2 mM tris pH 7·3 and purified by extraction in 0·6M KCl–5mM histidine, pH 7·3. The final sediment was resuspended in 40% sucrose-2 mM tris pH 7·3 to maintain a good uptake after several days (Repke & Katz, 1969). Preparations not older than 10 days were used.

Incubation was at 26° with a standard mixture for the measurement of Ca^{2+} uptake containing: 50 mM tris–HCl pH 7·3, 1 mM $MgCl_2$, 50 μM $CaCl_2$, carrier free $^{45}CaCl_2$ 30 μM ethyleneglycolbis (2-aminoethyl)tetra-acetate (EGTA) pH 7·3; 0·5 mM ATP disodium salt and 4 mM potassium oxalate. The protein concentration (Lowry & others, 1951) was 0·1 mg/ml. The amount of calcium 45 taken up was measured by a Millipore filtration technique (Martonosi & Feretos, 1964).

RESULTS

The kinetics of calcium accumulation appear to show both papaverine and eupaverin increase the rate of Ca^{2+} uptake in the presence of oxalate (Fig. 1). Eupaverin appears to be about 10 times more active than papaverine (Fig. 2).

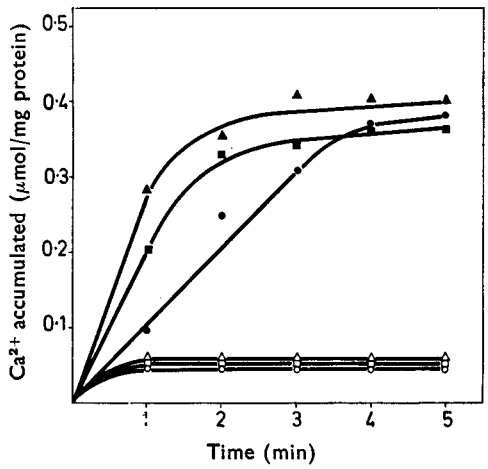

FIG. 1. Effect of papaverine and eupaverin on the velocity of Ca^{2+} uptake by sarcotubular vesicles in the absence and in the presence of 4 mM potassium oxalate. Experimental conditions are reported in the text. The reaction was started by the addition of microsomes at 0 time. (○) No potassium oxalate, (□) no potassium oxalate, 0·5 mM papaverine; (△) no potassium oxalate, 50 μM eupaverin; (●) 4 mM potassium oxalate; (■) 4 mM potassium oxalate, 0·5 mM papaverine; (▲) 4 mM potassium oxalate, 50 μm eupaverin.

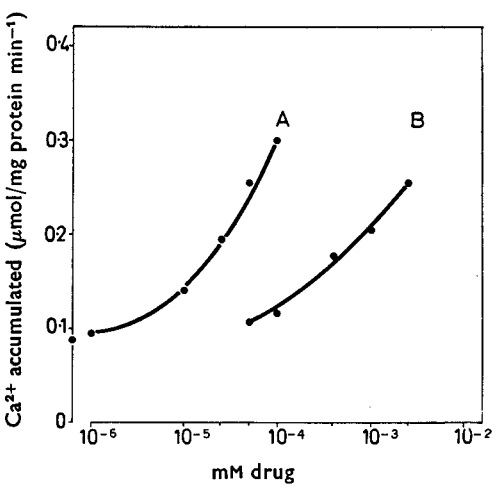

FIG. 2. Dose-response relation of Ca^{2+} uptake to papaverine HCl and eupaverin HCl in the presence of 4 mM potassium oxalate. Experimental conditions as in Fig. 1. Time of incubation 1 min. (●) eupaverin HCl; (○) papaverine HCl.

In Fig. 3 is reported the influence of various parameters affecting the rate of Ca^{2+} uptake in the absence and in the presence of 50 μM eupaverin. It appears that increasing concentrations of ATP (Fig. 3A) or oxalate (Fig. 3B) enhance the rate of Ca^{2+} uptake and that eupaverin induces a further increase of the velocity of this process. The increase of Mg^{2+} concentration reduces (Fig. 3C) the rate of Ca^{2+} uptake, in a parallel way, both in the control and in the presence of eupaverin, without significantly affecting the activity of the drug. Increasing concentrations of Ca^{2+}

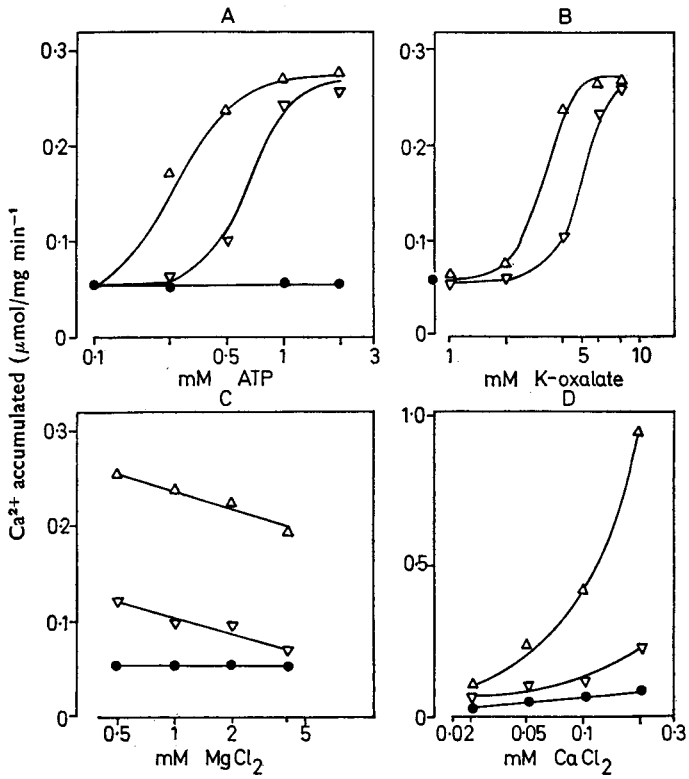

FIG. 3. Effects of varying concentrations of ATP, potassium oxalate, $MgCl_2$ and $CaCl_2$ on Ca^{2+} uptake in the absence and in the presence of 50 μM eupaverin HCl. Time of incubation: 1 min. (●) No potassium oxalate; (○) 4 mM potassium oxalate, (△) 4 mM potassium oxalate, 50 μM eupaverin.

(Fig. 3D) poorly enhance the velocity of uptake but clearly increase the activity of eupaverin.

The results obtained with papaverine 0·5 mM closely parallel those reported for eupaverin.

DISCUSSION

The results indicate that papaverine and eupaverin significantly enhance the rate of Ca^{2+} uptake by isolated sarcoplasmic vesicles and that this effect is influenced by the concentrations of ATP, oxalate and Ca^{2+}.

The degree of activity appears remarkable, especially for eupaverin, which was about ten times more effective than papaverine.

Since the sequestration of Ca^{2+} by sarcoplasmic reticulum is generally considered as the basic mechanism of relaxation in skeletal muscle (Ebashi, 1961; Hasselbach, 1964; Bianchi, 1970), the results of present investigations suggest that the increase of the rate of Ca^{2+} uptake by sarcoplasmic vesicles could play a part in the mechanism of the inhibitory effect on potassium and caffeine-induced contractures of frog skeletal muscle observed with papaverine and eupaverin (Buttar, 1969; Carpenedo: unpublished observation). No explanation can be obtained from these findings to account for the contracture elicited by high doses of these drugs.

The mechanism by which calcium is taken up, stored and released in the fibres of smooth muscle has not yet been elucidated (Hurwitz & Joiner, 1969). Also the presence of sarcotubular-like structures is still uncertain: in some types of smooth muscle it is conceivable that the surface membrane takes over the major function of the sarcoplasmic reticulum (Bianchi, 1970).

Isolated sarcoplasmic vesicles with the properties of binding calcium ions and of splitting ATP have been reported, but only for the cow uterus (Carsten, 1970). However, if, in smooth muscle, calcium sequestration and the consequent muscle relaxation develops through a process that is sensitive to papaverine and eupaverin, the enhancement of the rate of Ca^{2+} uptake by these drugs could be relevant in the mechanism of their spasmolytic activity.

No definite conclusions can be drawn about the mechanism of action of the drugs tested on calcium uptake; but cyclic AMP facilitates the uptake of Ca^{2+} by sarcoplasmic vesicles (Shinebourne & White, 1970) and papaverine and eupaverin strongly inhibit phosphodiesterase of various tissues (Kukovetz & Pöch, 1970; Markwardt & Hoffman, 1970; Triner, Vulliemoz & others, 1970).

Provided that isolated vesicles retain adenylcyclase and phosphodiesterase activities (Rabinowitz, Desalles & others, 1965; Toson & Carpenedo, unpublished) these drugs could modify calcium uptake by increasing cyclic AMP content of microsomes.

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