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Effects of some spasmolytic agents on the lipid-facilitated transport of calcium ions

SIR,—Depolarization of smooth muscle cells is accompanied by an influx of calcium ions. Woolley (1963) proposed that lipids were involved in the transport of calcium ions and phospholipids extracted from skeletal and cardiac muscle and nervous tissue were found to facilitate the transport of calcium ions from an aqueous to the chloroform phase (Feinstein, 1964; Nayler, 1966a). Several substances were found to elicit effects on this simple system consistent with their pharmacological properties (Feinstein, 1964; Blaustein & Goldman, 1966; Nayler, 1966a,b; Sandow & Isaacson, 1966; Blaustein, 1967; Piccinini & Pomarelli, 1967). We (Santi, Ferrari & Contessa, 1964; Tóth, Ferrari & others, 1966) implicated two effects in the mechanism of action of spasmolytics: (i) the inhibition of oxidative phosphorylation shared by papaverine and its main oxy-alkyl-benzylisoquinoline derivatives, and (ii) interference with calcium ions presumably assuming a prominent importance in the myolytic activity of other compounds devoid of inhibitory effects on energy production (Ferrari & Gaspa, 1965; Tóth & others, 1966). We now describe the effects of some myolytic agents on lipid-facilitated calcium transport.

Phospholipids were extracted (1 hr) from a homogenate of calf stomach muscle with chloroform–methanol (2:1) solution (1·5 ml)/g wet weight (Feinstein, 1964; Blaustein, 1967). The extract was washed and diluted with chloroform–methanol (2:1) to obtain a phospholipid concentration of 1·5 mg/ml (Folch, Lees & Stanley, 1957). The drugs (papaverine hydrochloride, eupaverin hydrochloride, isoxsuprine hydrochloride and aminopromazine hydrochloride) were dissolved at concentrations ranging from 0·1 to 2 mM in a medium containing 116 mM NaCl, 2·5 mM KCl, 0·5 mM $CaCl_2$, 0·2 μc/ml $^{45}CaCl_2$. Samples (0·5 ml) of this solution were added to 1 ml of chloroform–methanol phospholipid extract; the mixture was shaken for 1 min in a cyclomixer and then centrifuged for 10 min at 2500 *g*. Aliquots of 0·2 ml of the chloroform phase were tested for radioactivity in an end window Geiger counter.

Under these experimental conditions it was observed that aminopromazine, papaverine, eupaverin and isoxsuprine inhibit the lipid-facilitated calcium transport from the aqueous to the chloroform phase. Aminopromazine is the

TABLE 1. EFFECTS OF VARIOUS CONCENTRATIONS OF SPASMOLYTIC DRUGS ON THE LIPID-FACILITATED TRANSPORT OF CALCIUM IONS FROM AN AQUEOUS TO CHLOROFORM PHASE

Drugs	Concentration (mM)	% inhibition (± s.e.) of the lipid facilitated calcium transport
Papaverine hydrochloride	0·5	27·00 ± 2·06
	1·0	57·20 ± 1·28
	1·5	85·00 ± 3·07
	2·0	92·40 ± 0·60
Eupaverin hydrochloride	0·5	26·60 ± 0·73
	1·0	64·00 ± 5·90
	1·5	89·30 ± 4·10
	2·0	96·60 ± 3·30
Isoxsuprine hydrochloride	0·5	19·90 ± 2·90
	1·0	39·50 ± 5·00
	1·5	57·50 ± 0·03
	2·0	70·40 ± 2·32
Aminopromazine hydrochloride	0·1	8·00 ± 4·37
	0·2	32·50 ± 2·50
	0·5	91·50 ± 3·50
	1·0	100·00 ± 0·00

most effective compound: its activity is about four times greater than that of papaverine and eupaverin, which are more active than isoxsuprine (Table 1).

There seems to be a similarity of effects between the drugs tested and some local anaesthetics (Feinstein, 1964), and we suggest that like these anaesthetics, certain spasmolytic agents may interact with phospholipids, thus impairing their calcium binding ability. If phospholipids are effectively involved in calcium transport at membrane level, it seems of interest to mention the results of our previous investigations indicating that some myolytic agents prevent calcium uptake by red cell membranes (Carpenedo, 1966) and that a clear antagonism occurs between spasmolytic drugs and calcium ions both in polarized (Ferrari, 1964; Ferrari & Gaspa, 1965) and K-depolarized intestinal smooth muscle (Ferrari & Carpenedo, 1968).

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