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α-Amanitin prevents the positive inotropic effect of cardiac glycosides in-vitro

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We recently observed that cycloheximide and actinomycin prevent the positive inotropic effect of ouabain on atria and ventricle strips. On the other hand, they neither affect the force of contraction of the myocardium, nor modify the activity of other positive inotropic agents such as Ca^{2+} and noradrenaline (Arletti et al 1983). We inferred that either the synthesis of a short-lived protein is required for the inotropic effect of ouabain, or that at least part of its effect is due to the stimulated synthesis of some protein(s).

In an attempt to clarify this point we studied the influence of α-amanitin, which specifically inhibits mammalian DNA-dependent RNA polymerase (Fiume 1972), on the inotropic activity of ouabain and of digoxin.

Methods

Mongrel guinea-pigs (350–450 g) and rats of a Wistar strain (Morini, S.Polo d'Enza, Reggio Emilia, Italy) were decapitated and their hearts rapidly removed and placed in previously gassed (97% 0_2 −3% CO_2) standard Tyrode solution having the following composition (mM.)NaCl 115·0; KCl 4·7; $CaCl_2 \cdot H_2O$ 3·66; $MgSO_4 \cdot 7H_2O$ 1·2; KH_2PO_4 1·2; $NaHCO_3$ 25·0; glucose 10·0; pH 7·4. Strips (2·10 mm) of the right ventricle were mounted vertically in a double-chambered bath (volume = 20 ml) perfused at 32±0·2 °C with Tyrode.

One end of the ventricle strips was secured to the bottom of the chamber by means of platinum electrodes and the other end was attached to a force-displacement transducer (MARB, Pistoia, Italy). Resting tension was set at 0·8 g, and maintained at the same value for the duration of the experiment. The preparations were stimulated electrically at a constant rate (1 Hz) by square-wave pulses, of 5 ms duration, that exceeded threshold voltage by 20%. Pulses were delivered by a pulse generator (WP1 301–105; New Haven USA) connected to an isolation unit. The signal from the transducer was recorded via a dc preamplifier (MARB, Pistoia, Italy) on an oscilloscope monitor.

Experiments started after a 1 h equilibration period. Ouabain and digoxin (Aldrich-Europe, Beerse, Belgium) were diluted with Tyrode solution to the desired concentration and left in the bath for 1 h. α-Amanitin (gift of Prof. Th. Wieland, Max-Plank Institut, Heidelberg) was also dissolved in Tyrode and added to the perfusion medium 5 min before glycosides. Student's *t*-test was used for the statistical analysis of data.

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Results and discussion

Table 1 show that α-amanitin inhibits the inotropic effect of ouabain and of digoxin in a dose-dependent fashion. On the other hand, α-amanitin does not itself affect the force of contraction of the ventricle, even when present in the perfusion medium for 1 h.

The precise mode of action of cardiac glycosides is still unclear (Waser et al 1981), and there is at present no generally accepted theory to explain it (Akera 1981).

While it is widely agreed that these drugs specifically bind to the membrane Na, K-ATPase and that their toxic effects are due to inhibition of this enzyme (Repke 1963; Skou 1965; Akera et al 1970; Dunham & Gum 1972; Dahl & Hokin 1974; Wallick et al 1977; Brody et al 1977; Okita 1977; Akera & Brody 1978; Akera 1981; Erdmann 1981) with consequent calcium overloading (Gervais et al 1977; Nayler & Williams 1978; Nayler & Noack 1981), a growing body of evidence (Okita 1977; Nayler & Noack 1981; Klein & Evans 1961; Klein 1963; Dal Pra et al 1970; Cohen et al 1976; Besch & Watanabe 1978; Godfraind 1981) is in contrast with the hypothesis (Akera & Brody 1978; Wallick et al 1977; Brody & Akera 1977; Akera 1981; Erdmann 1981) that the same mechanism is responsible for their positive inotropic effect.

Table 1. Influence of α-amanitin, added to the perfusion medium 5 min before, on the positive inotropic effect of digoxin and ouabain. Rat and guinea-pig ventricle strips (2 × 10 mm) driven at 1 Hz.

Preparation	α-Amanitin concn M	Glycoside concn M	Max % increase in the force of contraction†
Rat ventricle-strip	$4 \cdot 10^{-8}$‡	—	6·30 ± 1·90 (11)
,, ,,	—	Ouabain, $1 \cdot 10^{-6}$	50·60 ± 5.10 (11)
,, ,,	$4 \cdot 10^{-8}$	Ouabain, $1 \cdot 10^{-6}$	11·50 ± 3·90 (6)
,, ,,	$2 \cdot 10^{-8}$	Ouabain, $1 \cdot 10^{-6}$	20·90 ± 2·90 (6)*
,, ,,	$1 \cdot 10^{-8}$	Ouabain, $1 \cdot 10^{-6}$	35·40 ± 1·50 (6)
Guinea-pig ventricle-strip	$4 \cdot 10^{-8}$**	—	5·90 ± 3.20 (12)
,, ,,	—	Ouabain, $3 \cdot 10^{-7}$	147·90 ± 29.70 (9)
,, ,,	$4 \cdot 10^{-8}$	Ouabain, $3 \cdot 10^{-7}$	58·80 ± 9.80 (6)**
,, ,,	—	Digoxin, $3 \cdot 10^{-7}$	217·50 ± 38.20 (6)
,, ,,	$4 \cdot 10^{-8}$	Digoxin, $3 \cdot 10^{-7}$	96·70 ± 23.40 (6)**
,, ,,	—	Digoxin, $1 \cdot 10^{-7}$	165·20 ± 29.60 (6)**
,, ,,	$4 \cdot 10^{-8}$	Digoxin, $1 \cdot 10^{-7}$	56·45 ± 10.23 (6)

† In parentheses the number of experiments.
‡ α-amanitin, alone, was left in the perfusion medium for 1 h.
* $P < 0.01$, ** $P < 0.02$, compared with controls (ouabain or digoxin alone) (Student's *t*-test).

Some investigators (Godfraind & Ghysel-Burton 1977; Ghysel-Burton & Godfraind 1979; De Pover & Godfraind 1979; Godfraind 1981) have postulated that there are at least two receptor sites for cardiac glycosides in myocytes: one associated with their positive inotropic action, and another (which is very likely part of the Na,K-ATPase molecule and whose occupation inhibits the activity of this enzyme) associated with their toxic effect. Indeed, it has been shown that the alteration by quinidine of the pharmacokinetics of digoxin and digitoxin involves mutual binding sites which are unrelated to Na,K-ATPase, while it increases digoxin and digitoxin toxicity by shifting them from the non-ATPase binding sites to the sites on Na,K-ATPase (Kim et al 1981). And in fact quite recently two distinct positive inotropic sites for ouabain have been found in rat ventricular strips. The higher-affinity response correlates with an apparently high-affinity site which can be detected by [^{3}H]ouabain binding to intact rat ventricular myocytes. These higher-affinity sites do not correlate with the concentration of ouabain necessary to inhibit Na,K-ATPase activity of sarcolemma preparation from rat ventricles, suggesting that in the rat ventricle the high-affinity site for the inotropic effect of ouabain may not be related to inhibition of Na,K-ATPase (Adams et al 1982).

Our present and recent (Arletti et al 1983) results, showing that inhibition of protein synthesis prevents the positive inotropic effect of ouabain, may suggest for cardiac glycosides a novel and so far unforseen mechanism of action.

On the other hand, control of the rate of synthesis of proteins is a mechanism of action common to steroid drugs and hormones (androgens, oestrogens, progestins, anabolic steroids, adrenocortical steroids and synthetic derivatives) (Schutz et al 1975; Chan & O'Malley 1976; Gorski & Gammon 1976; Iynedjan & Hanson 1977; Mainwaring 1977) and also to secosterols (De Luca 1976).

Since α-amanitin specifically inhibits DNA-dependent RNA polymerase in mammalian cells, the results of the present study seem to indicate that the positive inotropic effect of cardiac glycosides is, at least in part, linked to the stimulated synthesis of some protein(s) by an action at the transcriptional level.

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