Biochemical Pharmacology, Vol. 26, pp. 381–388. Pergamon Press, 1977. Printed in Great Britain.

INFLUENCE OF HIGHLY UNSATURATED PHOSPHATIDYLCHOLINE ON THE EFFECTS OF OUABAIN AND SOME CARDIOACTIVE DRUGS ON CARDIAC CONTRACTILE FORCE AND NA^+,K^+-ATPase ACTIVITY

HELMUT ENGEL, DIETFRID PROPPE and OTMAR WASSERMANN*

Departments of Pharmacology and Toxicology, University of Kiel, Hospitalstraße 4–6, D-23 Kiel, Germany

(*Received* 17 *June* 1976; *accepted* 7 *September* 1976)

Abstract—Isolated left guinea pig auricles and cardiac Na,K-ATPase from calf heart were incubated with highly unsaturated phosphatidylcholine (PC) for 2 hr. Thereafter the actions of ouabain on Na, K-ATPase, and of ouabain, digoxin, digitoxin, isoproterenol, acetylcholine, pentobarbital and different extracellular Ca^{2+} concentrations on contractile force of the auricles were investigated. PC itself altered neither the ATPase activity nor the intracellular ionic homeostasis, nor contractile force of the auricles. Similarly, the staircase phenomenon, the contractile response to different extracellular Ca^{2+} concentrations, to pentobarbital and ouabain, and the extent of ouabain-induced inhibition of the ATPase were not influenced. In addition the toxicity of ouabain was not altered. However, the rate of development of the ouabain-induced ATPase inhibition, as well as of the positive inotropism and toxic effects of ouabain, digoxin and digitoxin was markedly reduced. The maximum inotropic effect of digoxin and digitoxin, and the toxicity of digitoxin were significantly enhanced. Remarkably, the binding of [^{3}H]digitoxin was significantly diminished in PC-pre-incubated auricles, binding of [^{3}H]ouabain, however, remained unchanged. The dose–response curves to isoproterenol and acetylcholine, the latter also in the presence of physostigmine, were markedly parallel-shifted to the right. The modifying effect of highly unsaturated PC on the action of the drugs studied is suggested to be due to an altered physico-chemical state (fluidity) of the outer surface of the cellular membrane.

Numerous investigators have provided evidence that a close functional and conformational interdependency exists between the constituents of cellular membranes, i.e. proteins and lipids [3, 8, 15, 22, 25, 30, 60, 61]. Microarchitecture and functional state of many membrane-bound enzyme proteins are dependent on the presence of specific phospholipids which are attached to the protein in the form of an annulus. This has been proven for example with Ca-ATPase [50, 80, 81], Na,K-ATPase [10, 63], adenylate cyclase [36] and phosphotransferase [65] (for review, see 11a). A decrease in the amount of specific phospholipids associated with the protein or an exchange with different phospholipids will alter the enzymatic properties [10, 18, 63, 74, 79, 84].

The membrane-located Na,K-ATPase which is discussed as specific binding site for cardiac glycosides [5, 17, 19, 62, 69, 71], also belongs to the type of phospholipid-dependent protein. The enzymatic activity is entirely dependent on the presence of a specific phospholipid-annulus [10, 32, 63, 83]. The presence of these phospholipids influences essentially the binding of ouabain to the enzyme [10, 20].

In the present study, highly unsaturated phosphatidylcholine (PC) was allowed to interact with membrane lipids of a cardiac Na,K-ATPase preparation. Since the ouabain-induced inhibition kinetics of the enzyme were found to be altered, the question arose of whether phosphatidylcholine also might influence the effects of cardiac glycosides as well as their binding to cardiac tissue in isolated heart muscle preparations.

Additionally, under identical conditions the actions of Ca^{2+}, pentobarbital, acetylcholine and isoproterenol have been investigated.

Compounds. Digoxin, digitoxin (Boehringer, Mannheim), isoproterenol, physostigmine (Boehringer, Ingelheim), ouabain, $CaCl_2$, L(+) ascorbic acid (Merck, Darmstadt), pentobarbital (Knoll, Ludwigshafen) and acetylcholine iodide (Schuchardt, München) were used.

[^{3}H]ouabain (sp. act. 1mCi/0.05 mg) and [^{3}H]digitoxin (1mCi/0.038 mg) were purchased from NEN (Boston, Mass.) The radiochemical purity of the drugs was checked by thin-layer chromatography (TLC, 0.25 mm layer of silica gel 60_{254}, Merck, Darmstadt). The solvent systems were cyclohexane/acetone/acetic acid (49/50/1 and 65/33/2, v/v/v) to separate digitoxin and possible derivatives, and methanol/chloroform/water (45/45/5) in order to trace ouabain. The radioactive spots of the chromatograms were monitored by means of a radiochromatogram scanner (Packard model 7201/385) and compared with pure reference compounds. The radiochemical purity of the glycosides was greater than 98 per cent.

Highly unsaturated PC (Nattermann, Köln) of a mol. wt of about 800 was stored under nitrogen at low temperatures. The fatty acids of this PC consisted

* To whom reprint requests should be addressed.

of 85% of oleic, linoleic, and linolenic acids (16:0 12.5%, 18:0 2.6%, 18:1 9.0%, 18:2 70.1%, 18:3 5.8%).

The purity of the PC was thoroughly analysed according to the method of Walker [78] with TLC (0.25 mm layer of silica gel 60, Merck, Darmstadt). The solvent system was chloroform–methanol–water (65:35:5). The spots were identified by comparison with pure phospholipids (Serva, Heidelberg). The chromatograms were semi-quantitatively analysed by means of a densitometer (Vitatron) and by planimetry. The lysolecithin content of the PC amounted to less than 5 per cent.

ATPase. Na^+,K^+-activated, Mg^{2+}-dependent ATPase was prepared from calf heart ventricular muscle according to Matsui and Schwartz [48] including the purification with sodium iodide [54]. The enzyme preparation was deepfrozen in liquid air and stored at −20°. The protein content was determined by the method of Lowry *et al.* [39]. The enzyme activity was measured using the coupled enzymatic-optical assay [70] by means of a spectral photometer (Unicam SP 800). The sp. act. of the ATPase representing the total activity of the purified preparation including its ouabain-insensitive component was determined at 36° and pH 7.4 in a medium containing (mM) 100 NaCl, 10 KCl, 5 $MgCl_2$, 1 EDTA, 50 Tris, and amounted to 29 μmoles P_i/mg protein/hr. Maximal inhibition of the ouabain-sensitive portion occurred with 1×10^{-4} M ouabain and amounted to 92 per cent of the total activity.

In order to investigate the influence of PC on ATPase activity the enzyme was suspended for two hours at 0° in the aforementioned medium containing additionally 0.01% PC. Prior to addition, the PC was sonicated at 150 W for 3 min in an ice bath. Thereafter the enzyme activity was determined at 36°, and was found not to be affected by the presence of PC as compared to controls. At varying ouabain concentrations both the time to equilibrium of inhibition and the degree of inhibition of the ATPase activity were measured in the presence and absence of PC. PC revealed no measurable influence on the ATPase assay system activated by ADP in the absence of ATPase.

Isolated guinea pig auricles. Guinea pigs of either sex weighing 300–450 g body wt were used. The left auricles were dissected from the hearts and suspended in an organ bath.

Mechanograms of the isolated auricles were isometrically recorded by means of a strain gauge. The auricles were preloaded with 0.5 g and electrically stimulated by rectangular pulses (4 msec, 10–30 V, 3 Hz). The Tyrode solution (in mM: NaCl 137.0, KCl 2.7, $CaCl_2$ 0.9, $MgCl_2$ 1.0, $NaHCO_3$ 12.0, NaH_2PO_4 0.21, glucose 5.5) was oxygenated by a mixture of 95% O_2 and 5% CO_2, the temperature was maintained at 32°.

The auricles were equilibrated for 120 min in a Tyrode solution containing 0.1% PC sonicated as described above. During the equilibration period the incubation mixture was three times exchanged in order to minimize the formation of interfering degradation products of the phospholipids. Thereafter the auricles were incubated with phospholipid-free Tyrode solution. After an additional equilibration period of 10 min the drugs in question were added to the organ bath and the effects continuously monitored. The control auricles were equilibrated for the same time periods.

With respect to the effects of the cardiac glycosides special attention was focused on the rate of development of the positive inotropic effect and on the time to onset of toxic signs, i.e. increase in diastolic tension.

At the end of the equilibration period in both the controls and the PC-pre-incubated auricles the sodium and potassium content was determined by flame photometry. The auricles were blotted by a standard procedure, ashed in 1 ml of a mixture of equal amounts of $HClO_4$ (60%) and HNO_3 (65%), and re-dissolved in 0.1 n HCl. For each set of experiments the Na^+ and K^+ background was determined separately. The cellular Na^+ and K^+ content was corrected by subtracting the extracellular amount of Na^+ and K^+. The calculation was based on an extracellular space size (ECS) of 0.3 ml/g wet wt [9, 45].

After incubation with PC as described above the binding of the tritiated cardiac glycosides to the auricles was measured. After blotting the samples were dissolved in Soluene® 350 (Packard Instr.), and subjected to liquid scintillation counting. The concentration of the tritiated glycosides (in dpm) in the tissue (corrected for by the amount present in 0.3 ml ECS) relative to the medium concentration, was expressed as tissue/medium ratio

$$\frac{T}{M} = \frac{(\text{dpm/1 g tissue} - \text{dpm/0.3 ml medium}) : 0.7}{\text{dpm/1 ml medium}}.$$

Under identical conditions as described above for cardiac glycosides the influence of PC on the effect of Ca^{2+}, pentobarbital, acetylcholine and isoproterenol on the contractile force of the auricles was investigated. With respect to the correct calculation of the average dose–response curves, according to Ariëns *et al.* [2], the average concentration necessary to obtain a certain response was read from the individual dose–response curves (cf. Fig. 5).

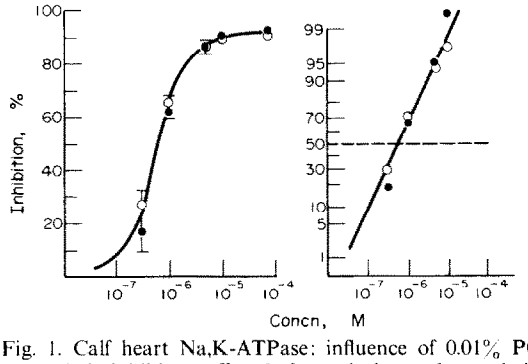

Fig. 1. Calf heart Na,K-ATPase: influence of 0.01% PC on ouabain inhibitory effect. Left graph: log molar ouabain concentrations (abscissa) are plotted against percent inhibition (ordinate) of the total activity of the enzyme including the ouabain-insensitive portion. Right graph: dose–inhibition curve of the ouabain-sensitive portion. The concentrations are plotted against percent inhibition on logarithmic-probability paper [37]. Half-maximum inhibition (ID_{50}) occurred with 5 to 6 $\times 10^{-7}$ M ouabain. ● PC-incubated; ○ controls; each point represents the mean of five individual experiments (±S.E.M., indicated as vertical bars, omitted if it lies in the magnitude of the symbols).

Electron microscopy. Right guinea pig papillary muscles which were phospholipid-pre-incubated and thereafter equilibrated in lipid-free Tyrode solution as described above were subjected to electron microscopy. No visible alteration of the cell membranes could be detected as compared to controls.

Statistics. The results were analysed for statistical significance by Student's *t*-test. The difference between mean values was taken as significant at $P < 0.05$ (P = probability of error).

Results

ATPase. After a two hr incubation period with PC the Na,K-ATPase activity and the extent of its inhibition by ouabain (Fig. 1) remained unchanged. However the time to equilibrium of inhibition was found to be significantly prolonged in the presence of PC (Fig. 2).

Isolated guinea pig auricles. In both the PC-incubated auricles and the control auricles the contractile force reached the same level and, due to ageing, decreased similarly during the equilibration period. (Table 1).

After a two hr equilibration period the auricles were subjected to a stepwise increase in stimulation frequency (1, 1.5, 2, 3, 4 Hz). Again, there was no difference between controls and PC-pre-incubated preparations when contractile force or the time required for adaptation of the contractile force to the altered stimulation frequency were compared.

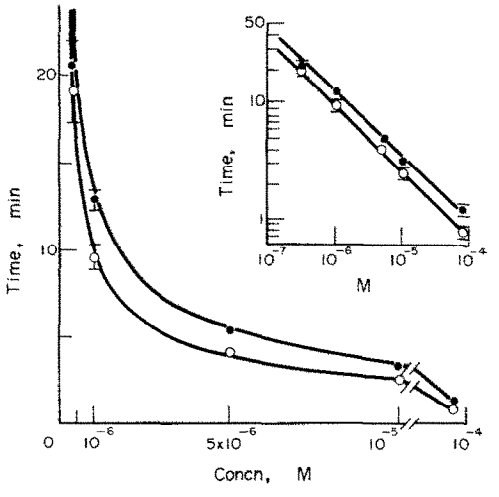

Fig. 2. Calf heart Na,K-ATPase: influence of 0.01% PC on time to equilibrium of ouabain-induced inhibition of the enzymatic activity. Molar ouabain concentration is plotted against time in min. Inset: log-log plot showing hyperbolic dependence of time to inhibitory equilibrium on ouabain concentration. Symbols, S.E.M. and number of trials as in Fig. 1. With the exception of the lowest concentration investigated all mean values differ significantly from each other ($P < 0.05$).

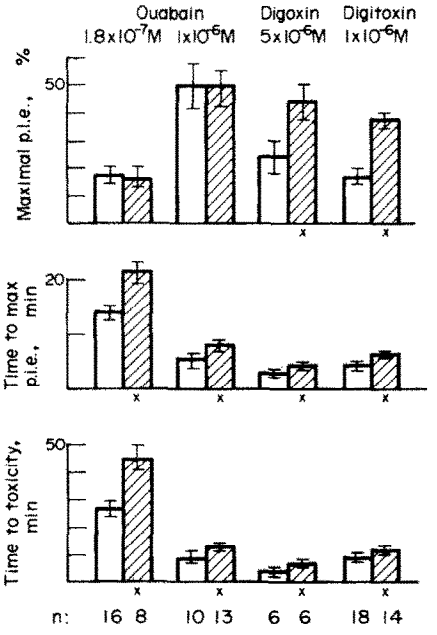

Fig. 3. Increase in contractile force and time to maximum positive inotropic effect (p.i.e.) and to the occurence of toxic symptoms induced by ouabain, digoxin, digitoxin in PC-pre-incubated left guinea pig auricles (striped bars). Mean ± S.E.M. * = $P < 0.05$.

With respect to the actions of ouabain, digoxin and digitoxin, the pre-incubation of the auricles with PC showed marked differences.

(1) The maximum positive inotropic effect of ouabain remained unchanged at non-toxic concentrations (1×10^{-7} M; increase in contractile force (mean ± S.D.) of controls 24 ± 15 per cent, $n = 8$, of PC-pre-incubated auricles 26 ± 12 per cent, $n = 9$; attained within 32 ± 6 min and 45 ± 12 min, respectively, $P < 0.05$), as well as at toxic concentrations (Fig. 3).

(2) Digoxin and digitoxin produced at all concentrations investigated a significantly larger maximum positive inotropic effect in PC-pre-incubated auricles than in controls (Fig. 3). This also held true for a non-toxic digitoxin concentration (6×10^{-8} M; increase of maximum positive inotropic effect from 23 ± 11 per cent, $n = 8$, to 39 ± 16 per cent, $n = 9$, $P < 0.05$; attained within 22 ± 3 min and 37 ± 9 min, respectively, $P < 0.05$).

(3) After PC-pre-incubation of the auricles the time courses of development of the effects of the three glycosides were significantly delayed (see above and Fig. 3, Table 2).

Table 1. Change in contractile force (%) of left guinea pig auricles during incubation with 0.1% PC. Mean ± S.D., $n = 8$

Incubation period (min)	0	15	30	45	60	90	120
Controls	100	90.4 ± 6.5	80.8 ± 6.1	79.1 ± 5.4	78.4 ± 7.9	74.9 ± 9.9	70.1 ± 9.9
PC-incubated	100	92.8 ± 8.7	84.9 ± 10.4	79.8 ± 11.4	78.9 ± 12.7	73.8 ± 13.0	72.2 ± 11.6

Table 2. Influence of 0.1% PC on time (min) to half-maximum positive inotropic response to ouabain, digoxin and digitoxin in left guinea pig auricles. Mean ± S.D., * = $P < 0.025$

M	ouabain 1×10^{-7}	ouabain 1.8×10^{-7}	ouabain 1×10^{-6}	digoxin 5×10^{-6}	digoxin 6×10^{-8}	digitoxin 1×10^{-7}	digitoxin 1×10^{-6}
Controls	14.68 ±3.05 $n = 8$	5.39 ±1.53 $n = 16$	2.59 ±0.43 $n = 10$	1.03 ±0.13 $n = 6$	10.32 ±3.95 $n = 8$	8.60 ±1.10 $n = 11$	1.67 ±0.43 $n = 16$
PC-pre-incubated	20.24* ±4.87 $n = 9$	7.44* ±2.05 $n = 8$	3.32* ±0.79 $n = 14$	1.48* ±0.28 $n = 7$	15.52* ±3.43 $n = 9$	13.26* ±2.48 $n = 13$	2.81* ±0.40 $n = 13$

In order to find out whether the increase of the digitoxin-induced positive inotropic effect in PC-pre-incubated auricles is accompanied by an increase in glycoside toxicity a digitoxin concentration of 1×10^{-7} M was investigated. In control auricles ($n = 12$) this concentration was not toxic. In 13 PC-pre-incubated auricles, however, 6 displayed toxic signs within one hour after glycoside administration.

In contrast, ouabain, which under similar conditions showed no enhanced inotropism in PC-pre-incubated auricles was not found to be more toxic in PC-pre-incubated auricles ($n = 11$) than in non-incubated ones ($n = 9$), using a threshold concentration of 1.2×10^{-7} M with respect to the occurrence of toxic symptoms.

Cellular sodium and potassium content. After the two hr equilibration period in both controls and PC-pre-incubated auricles the sodium and potassium content was in the normal range. Controls ($n = 5$): K^+ 87.5 ± 2.5, Na^+ 33.8 ± 3.6; PC-pre-incubated ($n = 5$): K^+ 90.3 ± 1.7, Na^+ 32.6 ± 1.8 (mean ± S.E.M. mmoles/kg cell).

Binding of [^{3}H]ouabain and [^{3}H]digitoxin to cardiac tissue. As demonstrated in Fig. 4, pre-incubation of the auricles with PC influenced neither the binding rate of ouabain (1×10^{-7} M) to cardiac tissue nor the maximum T/M ratio. In contrast, the uptake of digitoxin (0.6×10^{-7} M) by the auricles was delayed after PC-pre-incubation and the maximum T/M ratio was significantly reduced.

Influence of PC on the action of Ca^{2+}, pentobarbital, isoproterenol, and acetylcholine. Pre-incubation of the auricles with PC did not cause any change in the dose–response curves of Ca^{2+} and pentobarbital.

The dose–response curves of isoproterenol and acetylcholine obtained in PC-pre-incubated auricles, however, were significantly shifted to the right (Fig. 5).

The maximum effects of these four compounds were not affected by PC-pre-incubation.

Discussion

Phosphatidylcholine (PC) is one of the substantial constituents of cellular membranes [13, 57]. In PC of mammalian cell membranes saturated fatty acids like palmitic or stearic acid are predominantly esterified at C_1 of glycerol. At C_2 mainly unsaturated fatty acids occur like oleic, linoleic or arachidonic acid [52, 76]. The degree of saturation of the fatty acids influences essentially permeability, conductility, density, consistency, enzymatic reactions and other properties of the cell membrane [3, 8a, 12, 22, 32a, 46, 72a, 73].

In contrast to the physiologically occurring membrane-located phosphatidylcholines the PC used in the present study contained mainly the unsaturated linoleic acid. The incorporation of such a phospholipid [68], which enhances the amount of unsaturated fatty acids among the lipd components of the cell membrane, will eventually lead to an enhanced fluidity of the membrane, i.e. a looser molecular packing [6, 12, 29, 46, 76].

During incubation of cell membranes with phospholipids (PL) the latter will rapidly be incorporated into and exchanged with membrane-located lipids of the outer layer at half-lives of several min as has been determined for PC [49], whereby incorporation of PC into the cell membrane seems to prevail over exchange [68]. An exchange of PL molecules from one side of the membrane to the other ("flip-flop") seems to be limited due to the asymmetric PL bilayer of the plasmalemma [49].

PC which at physiological pH does not interact with Na^+, K^+ or Ca^{2+} [11, 55, 58, 64] appears to be almost exclusively present in the outer layer of the cell membrane [21, 47, 77, 85], whereas the inside surface of the cell membrane is mainly composed of cation-binding PL such as phosphatidylethanolamine and phosphatidylserine (PS), the latter being a sub-

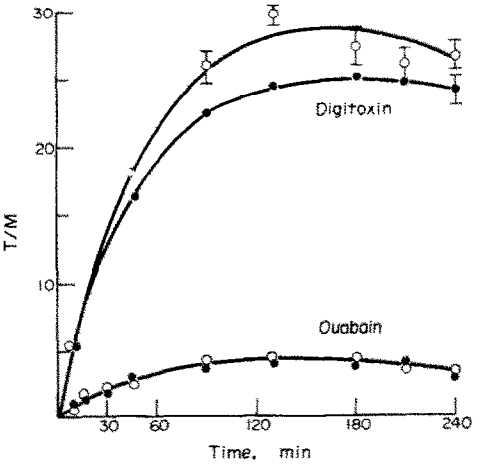

Fig. 4. Influence of PC-pre-incubation of left guinea pig auricles on [^{3}H]ouabain (1×10^{-7} M) and [^{3}H]digitoxin (0.6×10^{-7} M) binding to cardiac tissue. Ordinate: T/M ratio (radioactive glycoside per g cell divided by radioactive glycoside per ml Tyrode solution; see Methods). Abscissa: incubation period in min. ○ = controls, ● = PC-pre-incubated (mean ± S.E.M.). Each point represents the mean of at least five individual experiments.

stantial constituent of the membrane-located Na, K-ATPase [10, 20, 21, 47, 55, 58, 63, 64, 77, 84, 85]. At physiological pH, in polarized membranes Ca^{2+} binds highly specific to PS [42, 55a, 57, 73] thus forming rigid PS aggregates which will hardly be altered by PC molecules [56].

The interaction of PC with isolated cardiac muscle tissue influenced the effects of the drugs in very divergent ways. The findings may not be explained by unspecific impairment of the cell function: after a two hr incubation period with PC neither the cellular ionic homeostasis nor the development of contractile force were altered (Table 1), nor were the staircase phenomena (also with respect to the rate of adjustment to the altered frequencies) nor the inotropic response to increasing extracellular Ca^{2+} concentrations changed to a measurable extent. From the latter facts, including the unchanged maximum positive inotropic effect of ouabain (Fig. 3) which will be considered below, it may be inferred that PC had no influence on the binding characteristics of the intracellularly located binding sites for "coupling" calcium, nor on the fundamental processes involved in excitation-contraction (e.c.) coupling. This suggests that incorporation and exchange of the PC occurs essentially at the outer layer of the cell membrane.

Similarly, highly unsaturated PC neither affected the activity of the membrane-located Na,K-ATPase nor the binding of ouabain to cardiac tissue (Fig. 4). The polar compound ouabain seems to bind exclusively to extracellularly located specific binding sites of cardiac cell membranes [16, 43]. An intracellular accumulation of the radiolabelled drug as determined by means of autoradiography could not be proven [38]. In own experiments with auricles pre-loaded with digitoxin an unspecific binding of [^{3}H]ouabain was not detectable (to be published). An interaction with phospholipid membranes as has been proven for the non-polar digitoxigenin [66] is not to be assumed for the highly lipophobic ouabain. Similarly to digitoxigenin the non-polar digitoxin is estimated to bind specifically by only a few percent to cardiac muscle tissue [33] and to accumulate intracellularly ("unspecific" binding) [33, 34, 38, 43, 82].

According to several authors [5, 17, 19, 62, 69, 71] the specific binding site ("digitalis receptor") is referred to as the membrane-located Na,K-ATPase which binds ouabain, depending on the activity state of the enzyme [10, 23, 24, 72].

Interaction of cardiac glycosides with Na,K-ATPase seems to induce conformational changes beyond the protein of the membrane-bound enzyme [44, 53]. This change is thought to alter the characteristics of Ca^{2+} binding to the plasmalemma which will in turn improve the effectiveness of the e.c. coupling process and thus induce a positive inotropic effect [5, 41, 42]. In fact, some experimental evidence exists that cardiac glycosides might alter the calcium binding characteristics of the membrane-bound Na, K-ATPase [59, 71] as well as of Na,K-ATPase-containing cardiac sarcolemmal microsomes [41, 84a].

With respect to these interdependencies, the unchanged extent of the ouabain-induced positive inotropic and toxic effects in PC-pre-incubated auricles may suggest an unaltered affinity of the ATPase for ouabain and no influence of PC on the extent of ouabain-induced conformational changes. The unchanged extent of ouabain-induced ATPase inhibition seems to correlate to these interrelationships. This interpretation may be supported by the close positive correlation between cardioactivity of cardiac glycosides [4, 7, 40] and their potency to inhibit Na,K-ATPase [1a, 4, 26, 31, 75].

Remarkably, the inhibition of the ATPase, as well as the positive inotropic and toxic effects evoked by ouabain were correspondingly delayed by PC. Equally PC delayed the effects of digitoxin and digoxin (Fig. 3, Table 2). This delay may not be caused by a delayed formation of the glycoside ATPase complex, since PC-pre-incubation does not influence the binding of ouabain to cardiac tissue (Fig. 4). The fluidizing effect of the highly unsaturated PC could lead to a decreased interdependence between membrane proteins and lipids [3]. Possibly this may damp molecular re-arrangements, thus slowing down glycoside-induced conformational alterations of larger areas of the membrane.

The hydrophobic digitoxin binds to a large extent to unspecific binding sites, as indicated by the high T/M ratio (Fig. 4). After pre-incubation with PC the T/M ratio of digitoxin was found to be significantly reduced, the positive inotropic effect of the glycoside and its toxicity, however, were reinforced. A similar increase of the effects was found with digoxin (Fig. 3). The decrease of the digitoxin binding might be due to a decrease in unspecific binding, since (1) the positive inotropic effect ("specific" binding) did not decrease, but rather increased, and (2) a change in the "specific" binding would be lost in the large portion of non-specific binding.

Many speculations are possible with respect to the altered effects of digoxin and digitoxin following PC-pre-incubation of the auricles. A clear-cut explanation, however, cannot be given.

Alterations in the conformational interdependence between membrane proteins and lipids due to PC-interactions might also hold true for the specific binding sites of isoproterenol and acetylcholine. This may be taken from the dose–response curves of the two compounds which are considerably shifted to the right after PC-pre-incubation of the auricles (Fig. 5), indicating a reduced affinity of their binding sites or a handicap of the effector systems. (In the presence of physostigmine, the acetylcholine dose–response curve obtained after PC-pre-incubation was shifted to the right as well, indicating that PC did not affect the acetylcholinesterase). The functional integrity of these receptor proteins also requires the presence of phospholipids [14, 15, 27, 35, 36, 51]. Thus it is not surprising that the alteration of the physico-chemical properties of the membrane by interference with extraneous phospholipids like the PC under discussion modifies the normal receptor function.

In contrast to the compounds mentioned above, pentobarbital has no specific "receptor" at the cell membrane. Experimental evidence suggests that the drug enters the lipid compartments of the cell membrane [28], induces membrane expansion [67] and decreases the Ca^{2+} exchange rate [9], thus leading to a negative inotropic effect. In the case of pentobarbital PC failed to alter the dose–response curve. This

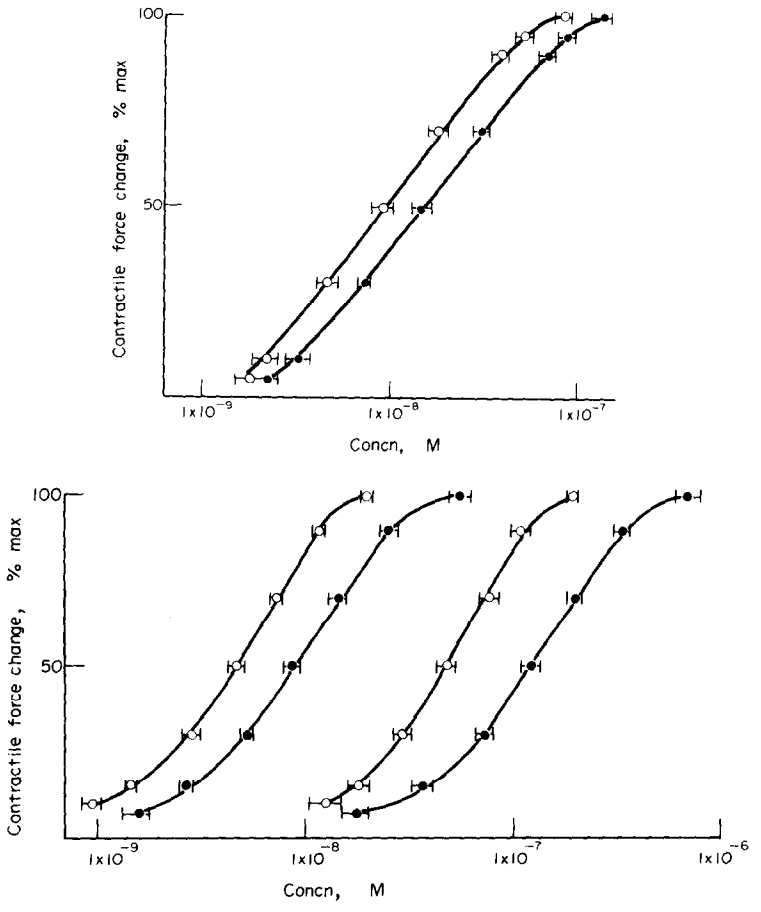

Fig. 5. Isoproterenol and acetylcholine log dose–response curves obtained on left guinea pig auricles after PC-pre-incubation. Upper panel: isoproterenol was added cumulatively to the organ bath containing Tyrode solution with 5×10^{-4} M ascorbic acid to stabilize isoproterenol. At this concentration the ascorbic acid did not influence the contractile force ($n = 6$). Lower panel: acetylcholine was given non-cumulatively in the absence (right pair of curves) and in the presence of 1×10^{-6} M physostigmine (left pair of curves). Ordinates: change in contractile force as percentage of the maximum effect. Abscissas: drug concentration in the organ bath. ○ = controls, ● = PC-pre-incubated (mean ± S.E.M.). Each point represents the mean of eight individual experiments. Concerning the calculation of the dose–response curves see Methods.

may be due to the fact that the action of this drug is not mediated by a complex proteo-lipid effector system, but by a more "unspecific" interaction with the membrane lipids, this interaction apparently being more or less independent of the fluidity of the membrane.

Concluding remarks

Pre-incubation with highly unsaturated PC did not influence the contractile response to different extracellular Ca^{2+} concentrations, the staircase phenomenon, the maximum positive inotropic effect of ouabain, the activity and ouabain-induced inhibition of the isolated Na,K-ATPase, as well as the ATPase-controlled cellular ionic homeostasis. Since the binding sites for coupling calcium as well as the phospholipid constituents required for the functional integrity of the Na,K-ATPase face intracellularly, these findings might indicate that the extraneous PC interacted only with the outer layer of the cell membrane.

The PC-induced delay of the glycoside effects both in isolated cardiac muscle and in isolated Na, K-ATPase may be explained by an increased fluidity of the cell membrane thus giving rise to a damping of molecular re-arrangements. On a similar basis the modifying effect of PC on the adrenergic and cholinergic receptors might be understood.

Acknowledgements—The expert technical assistance of Mrs Ursula Enslin and Mrs. Edeltraud Obst is gratefully acknowledged. The electron microscopic examination of the papillary muscles was kindly performed by Dr. R. Lüllmann-Rauch, Department of Anatomy, University of Kiel.

REFERENCES

1. T. Akera, D. Ku, T. Tobin and T. M. Brody, *Molec. Pharmac.* **12**, 101 (1976).
1a. J. C. Allen, M. L. Entman and A. Schwartz, *J. Pharmac. exp. Ther.* **192**, 105 (1975).

2. E. J. Ariëns, A. M. Simonis and J. M. van Rossum, in *Medicinal Chemistry* (Ed. G. de Stevens), Vol. 3: *Molec. Pharmac.*: The mode of action of biologically active compounds (Ed. E. J. Ariëns), Vol. 1, p. 119–286, Academic Press, New York–London (1964).
3. R. E. Barnett and J. Palazzotto, *Ann. N.Y. Acad. Sci.* **242,** 69 (1974).
4. G. G. Belz, M. Stauch, G. Belz, H. G. Kurbjuweit and A. Oberdorf, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.* **280,** 353 (1973).
5. H. R. Besch, Jr., and A. Schwartz, *J. mol. cell. Cardiol.* **1,** 195 (1970).
6. J. M. Boggs and J. C. Hsia, *Biochim. biophys. Acta* **290,** 32 (1972).
7. H. Böttcher, K. Fischer and D. Proppe, *Basic Res. Cardiol.* **70,** 279 (1975).
8. B. M. Braganca, in *Biomembranes; Architecture, Biogenesis, Bioenergetics and Differentiation* (Ed. L. Parker), p. 201, Academic Press, New York-London (1974).
8a. A. Bruni, P. W. M. van Dijck and J. de Gier, *Biochim. biophys. Acta* **406,** 315 (1975).
9. G. O. Carrier, H. Lüllmann, L. Neubauer and Th. Peters, *J. mol. cell. Cardiol.* **6,** 333 (1974).
10. A. R. Chipperfield and R. Whittam, *J. Physiol., Lond.* **230,** 467 (1973).
11. G. Colacicco, *Biochim. biophys. Acta* **266,** 313 (1971).
11a. R. Coleman, *Biochim. biophys. Acta* **300,** 1 (1973).
12. R. A. Demel, W. S. M. Geurts van Kessel and L. L. M. van Deenen, *Biochim. biophys. Acta* **266,** 26 (1972).
13. W. C. De Mello, in *Electrical Phenomena in the Heart* (Ed. W. C. De Mello), p. 89, Academic Press, New York-London (1972).
14. E. De Robertis, G. S. Lunt and J. L. la Torre, *Molec. Pharmac.* **7,** 97 (1970).
15. E. De Robertis, *Rev. Physiol. Biochem. Pharmac.* **73,** 9 (1975).
16. E. Erdmann and W. Hasse, *J. Physiol., Lond.* **251,** 671 (1975).
17. E. Erdmann and W. Schoner, *Klin. Wschr.* **52,** 705 (1974).
18. W. Fiehn and D. Seiler, *Experientia* **31,** 773 (1975).
19. T. Godfraind and M. Lesne, *Br. J. Pharmac.* **46,** 488 (1972).
20. S. S. Goldman and R. W. Albers, *J. biol. Chem.* **248,** 867 (1973).
21. S. E. Gordesky and G. V. Marinetti, *Biochem. biophys. Res. Commun.* **50,** 1027 (1973).
22. C. M. Grisham and R. E. Barnett, *Biochemistry* **12,** 2635 (1973).
23. O. Hansen, *Biochim. biophys. Acta* **233,** 122 (1971).
24. W. E. Harris, P. D. Swanson and W. L. Stahl, *Biochim. biophys. Acta* **298,** 680 (1973).
25. C. J. Hart, R. B. Leslie, M. A. F. Davis and G. A. Lawrence, *Biochim. biophys. Acta* **193,** 308 (1969).
26. K.-O. Haustein and J. Hauptmann, *Pharmacology* **11,** 129 (1974).
27. J. H. Hu, *Am. J. Physiol.* **223,** 882 (1972).
28. S. M. Johnson, K. W. Miller and A. D. Bangham, *Biochim. biophys. Acta* **307,** 42 (1973).
29. P. Joos and R. A. Demel, *Biochim. biophys. Acta* **183,** 447 (1969).
30. P. C. Jost, O. H. Griffith, R. A. Capaldi and G. Vanderkooi, *Proc. natn. Acad. Sci. U.S.A.* **70,** 480 (1973).
31. J. M. Kaufman and F. M. Belpaire, *Eur. J. clin. Pharmac.* **6,** 54 (1973).
32. H. K. Kimelberg and D. Papahadjopoulos, *Biochim. biophys. Acta* **282,** 277 (1972).
32a. H. K. Kimelberg and D. Papahadjopoulos, *J. biol. Chem.* **249,** 1071 (1974).
33. K. Kuschinsky, H. Lüllmann and P. A. van Zwieten, *Br. J. Pharmac.* **32,** 598 (1968).
34. K. Kuschinsky, H. Lüllmann and P. A. van Zwieten, *Br. J. Pharmac.* **34,** 613 (1968).
35. R. J. Lefkowitz, *J. mol. cell. Cardiol.* **7,** 27 (1975).
36. G. S. Levey, *J. mol. cell. Cardiol.* **4,** 283 (1972).
37. I. T. Litchfield, Jr., and F. Wilcoxon, *J. Pharmac. exp. Ther.* **96,** 99 (1949).
38. E. Löhr, H.-B. Makoski, T. Göbbeler and M. W. Strötges, *Arzneimittel-Forsch.* **21,** 921 (1971).
39. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193,** 265 (1951).
40. H. Lüllmann and Th. Peters, *Eur. J. Pharmac.* **14,** 204 (1971).
41. H. Lüllmann and Th. Peters, in *Recent Advances in Studies on Cardiac Structure and Metabolism*, Vol. 9, The Sarcolemma (Eds. P.-E. Roy and N. S. Dhalla), p. 311, University Park Press, Baltimore (1976).
42. H. Lüllmann and Th. Peters, *Clin. exp. Pharmac. Physiol.* **4,** 101 (1977).
43. H. Lüllmann, Th. Peters and U. Ravens, *Br. J. Pharmac.* **53,** 99 (1975).
44. H. Lüllmann, Th. Peters, J. Preuner and T. Rüther, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.* **290,** 1 (1975).
45. H. Lüllmann and P. A. van Zwieten, *Med. Pharmac. exp.* **16,** 89 (1967).
46. D. Marsh and I. Smith, *Biochim. biophys. Acta* **298,** 133 (1973).
47. S. D. Martay and K. H. Tsai, *Biochem. biophys. Res. Commun.* **65,** 31 (1975).
48. H. Matsui and A. Schwartz, *Biochim. biophys. Acta* **128,** 380 (1966).
49. M. G. McNamee and H. M. McConnell, *Biochemistry* **12,** 2951 (1973).
50. G. Meissner and S. Fleischer, *Biochim. biophys. Acta* **255,** 19 (1972).
51. D. M. Michaelson and M. A. Raftery, *Proc. natn. Acad. Sci. U.S.A.* **71,** 4768 (1974).
52. A. Montfoort, L. M. G. van Golde and L. L. M. van Deenen, *Biochim. biophys. Acta* **231,** 335 (1971).
53. K. Nagai, G. E. Lindenmayer and A. Schwartz, *Archs Biochem.* **139,** 252 (1970).
54. T. Nakao, Y. Tashima, K. Nagano and M. Nakao, *Biochem. biophys. Res. Commun.* **19,** 755 (1965).
55. S. Ohki, *Biochim. biophys. Acta* **282,** 55 (1972).
55a. S. Ohki and D. Papahadjopoulos, in *Surface Chemistry of Biological Systems* (Ed. M. Blank), pp. 155–174, Plenum Press, New York (1970).
56. S. Onischi and T. Ito, *Biochem. biophys. Res. Commun.* **51,** 132 (1973).
57. D. Papahadjopoulos, in *Biological Horizons in Surface Science* (Eds. L. M. Prince and D. F. Sears), p. 159, Academic Press, New York-London (1973).
58. D. Papahadjopoulos, *Biochim. biophys. Acta* **241,** 254 (1971).
59. D. Proppe, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.* **293,** R25 (1976).
60. W. R. Redwood, D. C. Gibbes and T. E. Thompson, *Biochim. biophys. Acta* **318,** 10 (1973).
61. W. R. Redwood and P. Weis, *Biochim. biophys. Acta* **332,** 11 (1973).
62. K. Repke and H. J. Portius, *Experientia* **19,** 452 (1963).
63. B. Roelofsen and L. L. M. van Deenen, *Eur. J. Biochem.* **40,** 245 (1973).
64. E. Rojas and J. M. Tobias, *Biochim. biophys. Acta* **94,** 394 (1965).
65. S. Rosemann, in *Biomembranes*, Vol. 2 (Ed. L. A. Manson), p. 9, Plenum Press, New York-London (1971).
66. R. Rösen, U. Fricke and A. Stier, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.* **293,** 39 (1976).
67. S. Roth and P. Seemann, *Biochim. biophys. Acta* **255,** 190 (1972).
68. C. J. Scandella, P. Devaux and H. M. McConnell, *Proc. natn. Acad. Sci. U.S.A.* **69,** 2056 (1972).

69. H. J. Schatzmann, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.* **263,** 12 (1969).
70. W. Schoner, C. von Ilberg, R. Kramer and W. Seubert, *Eur. J. Biochem.* **1,** 334 (1967).
71. A. Schwartz, *Biochem. Pharmac.* **25,** 237 (1976).
72. A. Schwartz, G. E. Lindenmayer and J. C. Allen, *Pharmac. Rev.* **27,** 3 (1975).
72a. J. Seelig and W. Hasselbach, *Eur. J. Biochem.* **21,** 17 (1971).
73. T. Seimiya and S. Ohki, *Biochim. biophys. Acta* **298,** 546 (1973).
74. H. Simpkins and L. E. Hokin, *Archs Biochem.* **159,** 897 (1973).
75. L. Storstein, in *Symposium on Digitalis* (Ed. O. Storstein), p. 158–168, Gyldendal Norsk Forlag, Oslo (1973).
76. L. L. M. van Deenen, *Naturwissenschaften* **59,** 485 (1972).
77. A. J. Verkleij, R. F. A. Zwaal, B. Roelofsen, P. Comfurius, D. Kastelijn and L. L. van Deenen, *Biochim. biophys. Acta* **323,** 178 (1973).
78. B. L. Walker, *J. Chromat.* **56,** 320 (1971).
79. J. A. Walker and K. P. Wheeler, *Biochim. biophys. Acta* **394,** 135 (1975).
80. G. B. Warren, P. A. Toon, N. J. M. Birdsall, A. G. Lee and J. C. Metcalfe, *Proc. natn. Acad. Sci. U.S.A.* **71,** 622 (1974).
81. G. B. Warren, M. D. Honslay, J. C. Metcalfe and N. J. M. Birdsall, *Nature, Lond.* **255,** 684 (1975).
82. P. G. Waser, *Experientia* **18,** 35 (1962).
83. K. P. Wheeler, *Biochem. J.* **146,** 729 (1975).
84. K. P. Wheeler, J. A. Walker and D. M. Barker, *Biochem. J.* **146,** 713 (1975).
84a. H. Will, B. Schirpke and A. Wollenberger, *Acta biol. med. germ.* **35,** 529 (1976).
85. R. F. A. Zwaal, B. Roelofsen and C. M. Colley, *Biochim. biophys. Acta* **300,** 159 (1973).