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NEW ASPECTS OF CARDIAC GLYCOSIDES

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PREFACE

When publication of the symposia held at the First International Pharmacological Meeting in Stockholm in August 1961 was discussed at the end of the meeting, it was generally agreed that speed should be the first consideration. It was therefore decided to include, besides the surveys presented by the invited speakers at the symposia, only a restricted number of short communications, selected according to their bearing on the topic of the symposium. The original plan of complete and detailed publication of the discussions, however, was abandoned, because it would inevitably have delayed publication. Some important contributions to the discussion, particularly those containing extensive experimental data, were to be presented in the form of a separate communication, others were left to the organizers for incorporation in the introduction. It is obviously unavoidable that selection and presentation of contributions in this form introduces a subjective element. My apologies are offered to those who feel that their contributions have not been incorporated adequately. I wish to conclude by thankfully acknowledging the most gratifying readiness and speed of collaboration on the part of all contributors, the great work they have done and the most valuable information they have conveyed to the meeting.

W. WILBRANDT

PREFACE

INTRODUCTION

W. WILBRANDT

Berne

THE main theme of the First International Pharmacological Meeting in Stockholm was the mode of action of drugs. Generally this question may be discussed on different levels: the organ level, the cellular and subcellular level and the molecular level. Furthermore actions of drugs may be therapeutic or toxic as far as the treated patient is concerned or they may be extratherapeutic, i.e. unrelated to therapy.

The cardiac glycosides, one of the most valuable groups of therapeutic agents at our disposal, have an interesting history. On the organ level they were first considered as diuretics, later as cardionotonics of particularly high organ specificity, devoid of direct diuretic potency. Recent work has provided unequivocal evidence that cardiac glycosides do have a renal action. The report of Professor Tanabe furnishes a valuable contribution in this respect. (The share of this action in the therapeutic diuresis, as distinct from secondary diuresis due to improvement of cardial performance, is a different question.) A more recent development is the discovery, in a vast variety of cells, of extratherapeutic actions (transport inhibition). This type of activity proved to be of surprisingly wide occurrence. In its mechanism, it may well be related to therapeutic effects.

The centre of interest, however, is still concerned with the therapeutic action on the heart, particularly on the contractile power of heart muscle. Considering the heart as a pump, utilizing various sorts of fuel, a first question arising is whether this effect is due to an increased supply of fuel energy, i.e. to stimulation of the energy yielding metabolism or to an effect on the pump machinery, i.e. on the contractile apparatus.

The discovery of actomyosin and its contraction *in vitro* has made a direct approach possible. In various types of muscle mod-

els, the energy supplying metabolism can be replaced by the addition of ATP and the reactions of the isolated contractile machine to cardiac glycosides can be studied. The reports of Professor Bing, Professor Waser and Doctor Lee, supplemented by contributions to the discussion particularly from Doctor Edman (related to his own work, see Ref. 4) deal with such studies. They indicate that binding of cardiac glycosides to actomyosin can be demonstrated *in vitro* by a variety of methods, and that contraction is affected by cardiac glycosides under certain conditions. The presence of calcium and of relaxing factor proved to be of particular importance.

Thus two sites of intracellular action appear possible: relaxing factor and actomyosin. With respect to actomyosin Professor Wollenberger pointed out in the discussion that according to Olson[6] in digitalized hearts the molar ratio glycosides: actomyosin was found to be in the order of 1 : 1000 or less. This objection is answered in Professor Waser's report by the assumption of a repeated action of one glycoside molecule on a large number of actomyosin molecules.

Effects of cardiac glycosides on the metabolism are mainly discussed in Professor Bing's report and considered further by Professor Repke and Doctor Lee. Assessment of possible direct actions of cardiac glycosides on metabolism is less easy than analysis of direct effects on the contractile system. Although changes of heart metabolism have been observed repeatedly, it is in general difficult to exclude—and in some observations it was possible to prove—that they are secondary to changes in activity or in internal conditions (e.g. ion concentrations). The fact remains that, with the exception of membrane ATPase, (see Professor Repke's report), no individual enzyme system could be identified as the site of a specific glycoside action. The general impression gained from the review of Wollenberger[12] appears unchanged: a primary effect on energy yielding metabolism is not likely to be the clue to the action of digitalis.

The effect of cardiac glycosides on membrane ATPase mentioned above should not be regarded as an exception to this statement. It is not a general effect on ATPase, but restricted to membrane (and/or microsome) ATPase and appears clearly related to the role of this enzyme in the cation transport across the cell membrane.

The discovery that cardiac glycosides inhibit ion transport initiated a new development at the cellular level. It is challenging in several respects.

The effect is mainly observed in transport systems operating "uphill" and therefore requiring energy (frequently termed "active transport"); yet it appears to be unrelated to the supply of energy. Its mechanism of action on the molecular level therefore seems to be intimately related to the transport mechanism proper and its study provides a valuable tool for the elucidation of such mechanisms.

Concerning the mode of therapeutic action of digitalis the problem arises as to whether the inotropic effect is the consequence of changes in ionic movements. Considering that other therapeutic (and toxic) actions, mainly those on the kidney tubules and on excitation and conduction in heart muscle, appear to be quite closely related to ion shifts, such an assumption would *a priori* not appear unlikely. Further questions then would be: which are the ion species involved, where and how do they act and which is the particular change in ion concentration characteristic for a therapeutic digitalis effect?

Much experimental work has been devoted to these problems in recent years. The reports of Professor Kahn, Professor Farah and Doctor Lee, partly that of Professor Repke and the communications of Doctor Klaus *et al.* and of Doctor Areskog deal with the results obtained. What is the present state emerging from these presentations?

It appears difficult to escape the conclusion that a clear answer is not yet available. Although there is much evidence, both in heart muscle and in other cells, to show that various ion concentrations may be affected by digitalis, in the myocardium such changes as expected from ion transport inhibition occur mainly in the range of toxic doses. This holds for the overall concentrations in heart muscle of potassium, calcium and sodium. These may now safely be considered ruled out as determining factors for the inotropic digitalis action.

Yet the parallelisms between this action and ion transport inhibition are so striking that it appears hardly possible to think of the two effects as unrelated phenomena. Pertinent observations are concerned with the range of active concentrations, with the antagonistic effect of potassium ions and particularly struc-

tural specificities (additional reference may be made to the data of Machova[5] published recently.)

The interesting results reported by Professor Repke on membrane ATPase may be considered as supplementary evidence along these lines, since an essential role of this enzyme in the membrane transport of cations appears convincingly established by the work of Dunham and Glynn[3] and particularly of Post *et al.*[8]

What, then, in view of the negative results as to critical ion concentration changes in heart muscle fibers, could be the basis of the relation between the inotropic and transport inhibitory effects of cardiac glycosides?

In the first place, it might be well to remember that heart muscle is not just a cell membrane bag containing ions and actomyosin, but a highly complex structure with different subcellular units arranged in a characteristic pattern as demonstrated most impressively by modern electronmicrographs.[9]

Before abandoning the ionic interpretation, efforts should therefore be made to rule out critical changes of local ion concentrations in subcellular spaces. Obviously this will not be an easy task. The reports of Professor Kahn and of Professor Farah and Professor Witt discuss possibilities along these lines.

A different possibility to account for the parallelisms mentioned that might be worth consideration would be the assumption that entrance of cardiac glycosides into the cell is in some way related to transport inhibition. Professor Repke's observation of a biphasic action of a cardiac glycoside on membrane ATPase is paralleled by some of the results on ionic changes in heart muscle reported by Dr. Kahn (Fig 10) and by Dr. Klaus *et al.* (Figs. 3 and 5), furthermore by observations on the frog skin potential.[11] These biphasic effects are reminiscent of recent observations in carrier transport systems.[10] Under suitable conditions competitive transport substrates in low concentrations may induce acceleration rather than inhibition of carrier transport. If cardiac glycosides are substrates of the cation transport system, they might be expected to exert such biphasic effects. It might be rewarding to submit this possibility to further experimental test. If it were substantiated, the essential action of cardiac glycosides might be intracellular and the

correlation between inotropic and transport inhibitory potencies might reflect differences in the affinity to a carrier receptor.

Observations possibly related to mechanismus along these lines are those reported by Doctor Moran, which show a striking dependence of uptake (and of action) of cardiac glycosides on the rate of stimulation in heart muscle.

Obviously the biphasic effects of cardiac glycosides on ATPase and on ion transport raise the further question: which of the two phases should be considered as related to the therapeutic action? An interesting suggestion was made by Doctor Schwartz in the discussion: could the stimulating phase be related to therapeutic, the inhibitory phase to toxic effects? If the two phases reflect the same intrinsic property of the molecule such an assumption would by no means be contradicted by the general correlation between transport-inhibitory and inotropic potencies. At present it is, however, speculative.

On the cellular level membrane ATPase and carrier system are closely related, both of them contributing to cation transport. On the molecular level, however, an effect on an enzyme molecule would appear to be a mechanism essentially different from that of a competitive carrier substrate.

The extensive studies on structure–action relationships surveyed by Professor Tamm and Profesor Chen reveal a considerable structural specificity. It appears difficult, however, at the present stage, to make use of the experiences in this field for the characterization of possible receptors.

It may be pointed out, however (as was done in the discussion), that the striking results obtained with ATPase by Professor Repke do not necessarily imply an effect on the protein molecule of the enzyme proper, nor do they exclude an action on the carrier system. The inhibition of ATP by cardiac glycosides concerns not the total enzyme activity but only the activation increment brought about by Na^+ and K^+. Post[7] interprets this "activation" as a consequence of the K^+ and Na^+ carrier transport cycle in the "enzyme" (which actually consists of membrane fractions and could not be purified without loss of the effects observed). Due to this transport the enzyme molecule on the internal side of the membrane is cyclically resupplied with its substrate, the supposed carrier. If this interpretation is accepted, the glycoside effect may well be on the carrier part of the

transport system rather than on the enzyme molecule. Actually observations in giant nerve axons appear not to be in harmony with a direct glycoside action on the ATPase enzyme molecule: cardiac glycosides act only when applied from outside, high energy phosphates only internally as reported by Caldwell *et al.*

The Stockholm symposium on cardiac glycosides certainly made it clear that a complete understanding of their action is not yet reached. Even on the organ level, recent years have brought new knowledge. On the cellular and molecular level certain approaches have opened up, but there are as yet more questions than answers. However, the meeting conveyed the encouraging impression of increasing contact between different fields and it was inspiring in suggesting new lines of attack.

REFERENCES

1. P. C. CALDWELL, A. L. HODGKIN, R. D. KEYNES and T. I. SHAW. The effects of injecting "energy-rich" phosphate compounds on the active transport of ions in the giant axons of Loligo. *J. Physiol.* **152**, 561–590 (1960).
2. P. C. CALDWELL and R. D. KEYNES. The effect of ouabain on the efflux of sodium from a squid giant axon. *J. Physiol.* **148**, 8P–9P (1959).
3. E. T. DUNHAM and I. M. GLYNN. Adenosinetriphosphatase activity and the active movements of alkali metal ions. *J. Physiol.* **156**, 274–293 (1961).
4. K. A. P. EDMAN. The action of ouabain on heart actomyosin. *Acta physiol. Scand.* **21**, 230–237 (1950). The action of ouabain on actomyosin from striated musculature, *Acta physiol. Scand.* **23**, 137–142 (1951); E. P. BARANY, K. A. P. EDMAN and A. PALIS. The influence of some factors on the rate of drop of viscosity in actomyosin-ATP-micture. *Acta physiol. Scand.* **25**, suppl. 89, 7–8 (1951); K. A. P. EDMAN, Action of cardiac glycosides on the ATP-induced contraction of glycerinated muscle fibers. *Acta physiol. Scand.* **30**, 69–79 (1954).
5. J. MACHOVA. Relationship between the effectiveness of cardiac glycosides in cats and their interference with red cell potassium transport. *Experientia* **16**, 553–554 (1961).
6. R. E. OLSON. Symposium on the physiology of cardiac muscle—introduction. *Amer. J. Med.* **30**, 649–652 (1961).
7. R. L. POST and C. D. ALBRIGHT. Membrane adenosine triphosphatase system as a part of a system for active sodium and potassium transport. In *Membrane Transport and Metabolism, pp.* 219–227. Sympos. Prag, Publ. House of the Czechoslovak Acad. Sci. (1961).
8. R. L. POST, C. R. MERRIT, C. R. KINSOLVING and C. D. ALBRIGHT. Membrane adenosine triphosphatase as a participant in the active transport of sodium and potassium in the human erythrocyte. *J. Biol. Chem.* **235**, 1796–1802 (1960).

9. R. J. STENGER and D. SPIRO. Structure of the cardiac muscle cell. *Amer. J. Med.* **30**, 653–665 (1961).
10. W. WILBRANDT and T. ROSENBERG. The concept of carrier transport and its corollaries in pharmacology. *Pharmacol. Rev.* **13**, 109–184 (1961).
11. W. WILBRANDT and E. M. WEISS. Antagonismus zwischen Herzglykosid und Corticosteroiden am Froschhautpotential. *Arzneimittelforschung* **10**, 409–412 (1960).
12. A. WOLLENBERGER. The energy metabolism of the failing heart and the metabolic action of the cardiac glycosides. *Pharmacol. Rev.* **1**, 311–352 (1949).