Biochemical Pharmacology, Vol. 24, pp. 823–827. Pergamon Press, 1975. Printed in Great Britain.

COMMENTARY

CARDIAC GLYCOSIDE RECEPTORS IN THE HEART

THÉOPHILE GODFRAIND

Laboratoire de Pharmacodynamie Générale, Université Catholique de Louvain, 4 Van Evenstraat, 3000 Louvain, Belgium

The term cardiac glycoside refers to any of the steroid glycoside compounds that have positive inotropic and characteristic electrophysiological effects on the heart. The more general term of digitalis is also used for these compounds although only some of them derive from the leaves of the common flowering plant known as foxglove or *Digitalis purpurea* or from *D. lanata*. The main drugs used therapeutically are digitoxin, digoxin and ouabain (obtained from the seed of *Strophantus gratus*). They are used to treat several cardiac disturbances but mainly congestive heart failure. Almost 200 years ago, William Withering described various therapeutic characteristics of digitalis and stated that it affected heart activity more profoundly than any other drug available at that time.

All cardiac glycosides contain a steroid nucleus to which an α, β-unsaturated lactone ring is attached at the C-17 position. The molecule without attached sugars is called the genin (or aglycone). Cardiac glycosides differ in substituent groups on the steroid nucleus as well as in the structure and number of sugars attached at the C-3 position. Molecular requirements for optimum biological activity include β-oriented α, β-unsaturated lactone at C-17, β-oriented hydroxyl groups at C-B and C-14 together with a *cis* A/B ring junction. The *cis* fusion of rings C/D of cardiac glycosides is not found in any other naturally occurring steroids [1].

Cardiac glycosides exert both cardiac and extracardiac effects [2]. This report will be restricted to the cardiac action and more specifically to the biochemical factors responsible for the positive inotropic effect. The recent growth of research on muscle contraction has emphasized the key role exerted by free cytoplasmic calcium in the contractile process [3, 4]. It has been known for a long time that the myocardial actions of calcium and digitalis are similar in certain respects and that the action of digitalis on the heart is dependent on the calcium concentration of the perfusion fluid [61]. It is therefore tempting to propose that the positive inotropic effect is the consequence of an enhanced increase of free cytoplasmic calcium due to an amplification of the coupling between excitation and intracellular response. From a biochemical point of view, several cellular systems may be the target sites for digitalis: the contractile machinery, the myocardial energy metabolism and the regulatory function of the plasma membrane and/or of the membranes of intracellular organelles.

At the present time, there is no clear demonstration of a direct action of digitalis on the physicochemical or on the biochemical properties of contractile proteins [5, 6]. Cardiac glycosides modify the oxygen consumption of isolated heart: low doses stimulate this consumption and high doses reduce it. The increased oxygen consumption is associated with an increased glycogenolysis. These metabolic changes are not related with an action of digitalis on an isolated and well defined enzyme system [5, 7]. They are only observed in intact tissues and require the integrity of the cell membrane. Another important effect of digitalis on the cell membrane is the well-known inhibition of the Na^+–K^+ exchange pump [8]. This inhibition has been described in most types of cells. It seems to represent the most specific action of digitalis, so that the question is open whether this inhibition does or does not represent the first step leading to several cell changes in response to digitalis, namely to the positive inotropic effect. To try to answer this question, I shall first consider the characteristics of the Na^+–K^+ pump.

THE CHARACTERISTICS OF THE Na^+-K^+ PUMP

For an ion moving across the cell membrane in a purely passive manner, provided there is no interaction between the ions, the ratio of influx to efflux can be calculated from the Ussing equation:

$$\frac{J_{1\to 2}}{J_{2\to 1}} = \frac{C_1}{C_2} \exp zEF/RT \qquad (1)$$

where $J_{1\to 2}$ and $J_{2\to 1}$ are unidirectional fluxes, C_1 and C_2 are the activities of the solutes on sides 1 and 2 respectively. For Na^+ the negative internal potential and the higher activity of Na^+ outside the cell should result in a flux ratio much greater than the observed value of unity. This provides evidence that the efflux of Na^+ cannot be purely passive and must involve some form of active transport. The system responsible for this transport is generally called the Na^+–K^+ pump or more simply the Na^+-pump. The properties of the Na^+-pump have been intensively investigated in red blood cells and nerves. The results obtained from these two dissimilar tissues are in complete agreement and suggest that the Na^+-pump is a constant feature of cell membranes. The main characteristics of the pump are: the dependence on ATP; the orientation in the membrane; the sensitivity to cardiac glycosides; and the reversibility.

In 1953, Schatzmann showed for the first time that cardiac glycosides inhibit the ionic restoration of red blood cells after cooling. Glynn (1957) demonstrated that cardiac glycosides were acting on the transport mechanism and not on the energy metabolism of the cell, he noticed also that the potency of cardiac glycosides was dependent on their molecular structure [8, 9].

In microsomes obtained from crab nerves, Skou discovered an ATPase activated both by Na^+ and K^+ [10]. Subsequent data have shown that this enzyme is intimately related to the Na^+-pump and is called the Na^+–K^+-ATPase or transport ATPase (ATP phosphohydrolase EC 3.6.1.3). In red blood cells, the activation of the enzyme obeys the same conditions as those of the Na^+-pump [11]. The enzyme is inhibited by cardiac glycosides and the ED_{50} for inhibition of the pump has the same magnitude as the I_{50} for the enzymic activity [9]. In resealed erythrocytes ghosts, it has been shown that the activation of the pump depends on $Na^+{}_i$ and $K^+{}_o$ [12]. When the ionic ratio is inverted, there is ATP synthesis showing that the pump is reversible [13]. Another argument in favour of the orientation of the system is the demonstration that ouabain is not active when it is injected intracellularly [63]. Studies on the stoichiometry of the Na^+-pump in terms of energy utilization indicate that 3 Na^+ are pumped per ATP hydrolyzed [14]. The ratio of the number of Na^+ pumped in exchange for K^+ varies between 1:1 and 3:2 depending upon the experimental conditions.

Finally it must be pointed out that Ca^{2+}-activated ATPases have also been found in microsomes which could be responsible for Ca^{2+} translocation; they are not sensitive to cardiac glycosides.

THE PROPERTIES OF Na^+–K^+-ATPase

Several authors have proposed hypothetical reaction mechanisms for the Na^+–K^+-ATPase. The most documented reaction mechanism is thought to consist of four sequential partial reactions [15]:

(1) In the presence of Na^+ and Mg^{2+}, Na^+–K^+-ATPase accepts the terminal phosphate group of ATP to form a phosphoenzyme.

(2) The second reaction is a magnesium-dependent change in the conformational state of the phosphorylated form.

(3) The third reaction is a potassium dependent hydrolysis of the second form of the phosphorylated intermediate.

(4) The fourth reaction is a spontaneous relaxation to the original conformation, accompanied by a loss of Mg^{2+}.

The evidence for this sequential transport model is derived from enzymatic and electrophoretic characterization of microsomal preparations and requires that a sodium-induced chemical change preceedes the potassium interaction.

Other authors have proposed a simultaneous rather than a sequential model. As pointed out by Lindenmayer *et al.* [16], in fragmented membrane preparations, competition between the cations for the two types of activation sites constitutes the greatest obstacle to derivation of kinetic models for Na^+–K^+-ATPase. Whittam and Chipperfield [17] have proposed a schematic model considering that:

(1) The binding site for Na^+ faces the inside and that for K^+ the outside; both can be occupied simultaneously. Na^+ and K^+ bind without ATP being required; this proposition is in agreement with Hansen and Skou [25].

(2) The second step is a phosphorylation with ATP + Mg^{2+} to form a transient, unstable complex in which there is an intramolecular shift in the position of the cation binding sites, such that they become oriented with K^+ facing the inside and Na^+ the outside.

(3) The dephosphorylation is a consequence of the formation of the complex which is not, in their view, an intermediate in the biochemical sense but in the chemical sense of a transient complex commonly postulated in the field of reaction mechanisms.

The simultaneous model proposed by Lindemayer *et al.* considers the existence of three equivalent sites for sodium and two nonequivalent sites for potassium. Their scheme is in agreement with allosteric or multiple-independent-site models. It is also in agreement with Albers and Koval but not with Robinson who has proposed that in the presence of Na^+ an alternative enzyme pathway, characterized by a higher affinity for K^+, becomes available through the phosphorylation of the enzyme by the substrate [18, 19]. Purification studies suggest that Na^+–K^+-ATPase preparations consist of lipoprotein particles that contain at least two proteins, one of which can be phosphorylated and another is a glycoprotein. The glycoprotein could act as a regulator of the pump. Studies by polyacrylamide gel electrophoresis in sodium dodecyl sulfate have shown that only one peptide component appeared to be common to the various preparations obtained from a variety of mammalian tissues. This peptide is the only constant component labeled by the sodium-dependent incorporation of phosphate from ATP. It has been shown that it contains at least one sulfhydryl group that can be alkylated by *N*-ethyl-maleimide [20–22]. The presence of -SH groups explains Bacq's earlier observation that so called thioloprive compounds have some similarities with cardiac glycosides [23].

THE BINDING OF CARDIAC GLYCOSIDES TO FRAGMENTED MEMBRANES

Studies of the binding of glycosides to Na^+–K^+-ATPase preparations were initiated by Matsui and Schwartz (1967). Their work and other subsequent studies [22] have demonstrated that the binding shows many features which are analogous to the pump action, namely the requirement for ATP and Mg^{2+}, the stimulation by Na^+ and the inhibition by K^+. There are however some differences as the nucleotide specificity is low whereas only ATP supports active Na^+ transport.

There are also some discrepancies among the various experimental reports describing the stability of the glycoside–enzyme complex and the similarity between $K^+{}_i$ and I_{50} [22, 24–27]. Possible explanations for these discrepancies are the various experimental conditions as well as the origin and nature of the preparation used. It is generally admitted that a conformational change must occur before ouabain can become bound; this change is achieved in the presence of different ligands (Mg^{2+} + ATP + Na^+), (Mg^{2+} + Pi), (Mn^{2+}), (Mg^{2+} + ATP), (Mg^{2+}). Although it has been shown that the rates of association and of dissociation are independent of the ligand [24], it has been reported by Whittam and Chipperfield [17], that the

binding with Mg^{2+} + ATP + Na^+ was dissimilar to that with Mg^{2+} regarding the effects of cations, inorganic phosphates (Pi) and pH. With Mg^{2+}, cations inhibited in the order Tl > K^+ > Na^+ > choline, but with Mg^{2+} + ATP + Na^+, only Tl and K^+ were inhibitory. Pi stimulated ouabain binding with Mg^{2+} but not with Mg^{2+} + ATP + Na^+. Ouabain binding with Mg^{2+} varied with pH like ATP hydrolysis but with Mg^{2+} + ATP + Na^+, the binding was constant from pH 5·0 to 8·3. It must be pointed out that some of the conformations which bind ouabain *in vitro* are not part of the natural reaction pathway: it seems unlikely that the conformation revealed by the effects of Pi is a step of the natural mechanism in view of the similar effects of phosphate and arsenate. The natural ligands are Mg^{2+} + ATP + Na^+. K^+ decreases the rate of association and of dissociation of the enzyme–ouabain complex; K^+ inhibition is not so simple as it depends on the glycoside concentration as well as on the concentrations of ligands.

THE BINDING OF CARDIAC GLYCOSIDES TO ISOLATED HEART

As early as 1967, it was recognized that cardiac glycosides were taken up from the perfusion medium by isolated heart muscle [28, 29]. Several attempts have been made to analyse the factors controlling this process as well as to localize and characterize the sites of the binding. Studies carried out using differential centrifugation of heart previously labelled with ^{3}H-glycosides, have shown that the drugs were preferentially bound to the microsomal fraction containing fragments of plasma membranes [30, 31]. Godfraind and Lesne [32] compared the uptake of various cardiac glycosides, digitoxin, ouabain, dihydro-ouabain, by isolated guinea-pig atria. The three compounds studied show certain dissimilarities. Digitoxin is a non-polar compound and shows high affinity for proteins, whereas ouabain is a polar compound with low affinity for proteins. Saturation of the lactone ring yields dihydro-ouabain and drastically reduces the positive inotropic action, dihydro-ouabain being 40 times less potent than ouabain in this respect [33]. The uptake of ^{3}H-ouabain and of ^{3}H-digitoxin by isolated atria is a complex process involving a saturable and a non-saturable binding site. It was found to fit the following equation:

$$U = aC_m + \frac{bC_m}{C_m + K_b} \qquad (2)$$

where U is the tissue concentration at equilibrium corrected for cardiac glycoside content of the inulin space, C_m is the cardiac glycoside concentration in the medium, a is the constant for the linear non-saturable uptake, b and K_b are capacity and equilibrium constants for the saturable binding site. Table 1 gives the parameters describing uptake of cardiac glycosides by guinea-pig atria, analysed following this model. The main difference between the two glycosides is in the clearance of the non-saturable site, which could represent a non-specific uptake; the saturable binding site may therefore be considered as the specific digitalis binding site. This view is favoured by the following observations:

Table 1. Computed estimates of parameters describing the uptake of cardiac glycosides by guinea-pig atria at 37° in Tyrode solution [32]

	Digitoxin	Ouabain
a (ml/g wet wt)	4·764 ± 0·046	0·100 ± 0·002
b (nmoles/g wet wt)	0·652	0·158
K_b (nmoles/ml)	208 ± 43	312 ± 44

(1) The uptake of inactive doses of dihydro-ouabain was only by the non-saturable mechanism.

(2) The uptake of labelled digitoxin and ouabain was reduced in the presence of another glycoside to the non-saturable uptake.

(3) At 4°, the tissue clearance of ouabain was similar to that of the non-saturable binding site and was not influenced by a large excess of digitoxin.

The dependence of specific binding on tissue metabolism is consistent with the observation that the presence of ATP is required for the activation of Na^+–K^+-ATPase and for the binding of cardiac glycoside to this enzyme [22]. Comparison of the concentrations of cardiac glycosides producing a half saturation of the saturable binding site with the ED_{50} for the inotropic effect, for inhibition of the sodium pump and for inhibition of Na^+–K^+-ATPase shows that all these values lie close together (Table 2), suggesting that a single receptor may be responsible for those actions of cardiac glycosides. On the basis of the present data, the receptor capacity for ouabain appears to be 9·5 × 10^5 molecules/cell. The density of Na^+–K^+ pumping sites in guinea-pig atria is about 300 sites per μm^2 membrane which can be compared with 750 for mammalian C fibers [35] and less than one for the red blood cells [36]. In guinea-pig atria, considering that the area covered by the genin is approximately 10^{-14} cm^2, 9·5 × 10^5 molecules of ouabain would cover about 0·034 per cent of the plasma membrane.

INTERACTION OF DIGITALIS WITH Na^+–K^+-ATPase AND THE PHARMACOLOGICAL AND THERAPEUTIC ACTIONS

Studies of the binding of cardiac glycosides to isolated heart strongly suggest that a single receptor might be responsible for the actions of cardiac glycosides and that this receptor is the Na^+–K^+-ATPase. As I have already mentioned, it is likely that the positive inotropic effect results from an enhanced increase in free cytoplasmic calcium occurring at each excitation. It has been observed that the calcium influx in guinea-pig atria increases when intracellular Na^+ (Na^+_i) increases [4]. This suggests the existence of a Na^+–Ca^{2+} transmembrane coupling mechanism [62] which has been demonstrated in nerve [38] and in smooth muscle [39]. The increased Na_i^+ due to the inhibition of the Na^+-pump could activate this mechanism, and could therefore be responsible for the increase in ^{45}Ca uptake evoked by digitalis. Another site of Na^+–Ca^{2+} interaction is the sarcoplasmic reticulum which is one of the intracellular calcium stores. It has been shown that the oxalate-dependent calcium uptake in sarcoplasmic reticulum vesicles obtained from cardiac [40], striated [41] or smooth muscle [42] is reduced when the incubation medium contains Na^+ instead of K^+. A possible site of Na^+–Ca^{2+} interaction could also

Table 2. Guinea-pig heart muscle. Comparison of the concentrations producing a half-saturation of the saturable binding site, 50 per cent diminution of ^{42}K uptake, 50 per cent of the maximum inotropic response of papillary muscle and 50 per cent inhibition of cardiac Na^+–K^+-ATPase. Concentrations are expressed as μM

Cardiac glycosides	Half-saturation (atria)	50 per cent inhibition of ^{42}K uptake	50 per cent inotropic effect (papillary muscle)	50 per cent inhibition of cardiac Na^+–K^+-ATPase
Digitoxin	0·208†	Atria 0·24† Ventric. 0·21†	0·28*	0·220‡
Ouabain	0·312†	Atria 0·60† Ventric. 0·35†	0·34*	0·630‡
Dihydro-ouabain	—	—	14*	24‡

* Data of Reiter [34].
† Data of Godfraind and Lesne [32].
‡ Data of Godfraind and De Pover [33].

be the mitochondria [43]. It has been observed that the inhibition of the Na^+-pump reduced the intracellular Ca^{2+} sequestering capacity of smooth muscle [39] and a similar observation has been repeated in guinea-pig atria (unpublished data). Such a mechanism could explain the increase in Ca^{2+} exchangeability preceeding the net increase in Ca^{2+} content of digitalis-treated hearts [3, 49]. These observations indicate that the relationship between the changes in the Na^+-pump activity and the cellular calcium content is far from being a simple one.

Some facts do not fit with the unique theory [50–54]. Among them are the observations made in experiments designed to compare the rate of onset or the rate of offset of parameters related with the inhibition of the Na^+-pump or the inhibition of the Na^+–K^+-ATPase and the positive inotropic effect; a difference in the kinetics of such parameters was considered as evidence against the existence of a unique receptor. In this respect, evidence was provided by Lüllmann and Ravens [51] who showed that the increment in contractile force evoked by cardiac glycosides in guinea-pig papillary muscles developed more rapidly than the reduction of the transmembrane action potential which is generally attributed to the inhibition of the Na^+-pump. From a biochemical point of view, the most demonstrative experiments are those of Okita *et al.* [50], in which they extracted Na^+–K^+-ATPase from hearts treated with cardiac glycosides. They compared, after several washout periods in drug-free solutions, the residual Na^+–K^+-ATPase activity with the inotropic effect; their main observation was the existence of a residual 39 per cent inhibition of the enzyme when the pharmacological effect had disappeared. Such observations conflict with other reports [55–60]; alternatively, however it might be postulated that the relationship between digitalis binding to the receptor and the subsequent biochemical and pharmacological effect is not under all circumstances a one-to-one process. Such a theory could also be invoked in order to take into account the therapeutic mode of action of digitalis.

The concentration of free cardiac glycosides in blood of patients under digitalis therapy is about 3×10^{-9} M. As recently demonstrated by Godfraind [2, 45] this concentration is too low to evoke any inhibition of the Na^+-pump of human heart although it does inhibit slightly the Na^+–K^+-ATPase. This indicates that digitalis could act by a mechanism which would not involve the inhibition of the Na^+-pump. It has been shown that therapeutic concentrations of digitalis do not evoke a positive inotropic effect *in vitro* but cause a post-synaptic potentiation characterized by an enhanced response of cardiac and smooth muscles to neurotransmitters [44–46]. This increase in muscle responsiveness is associated with changes in tissue ionic content which apparently are not compatible with an inhibition of the Na^+-pump as there is an increased uptake of ^{42}K [32]. We recently observed (unpublished observations) that when guinea-pig atria are incubated in Tyrode solution at 30° for 3 hr in the presence of ouabain 10^{-9} M, their K^+ content is increased, while their Na^+ content is decreased. Experiments with smooth muscle [45, 46] and experiments in progress using heart muscle indicate that therapeutic concentrations of ouabain increase the binding of Ca^{2+} to superficial high affinity binding sites, in agreement with experiments performed with higher doses under non equilibrium conditions [3, 47, 48].

CONCLUDING REMARKS

One of the points still not clear in the action of digitalis is the relationship between the binding to the receptor and the subsequent change in calcium binding to superficial sites. This question as well as the relation with the rate of calcium influx need to be clarified before coming to a final conclusion about the existence of a unique receptor for digitalis in the heart. The problem is not easy as the relationship between digitalis and Ca^{2+}-binding sites could be the result of a functional or a chemical (direct) interaction. If Na^+–K^+-ATPase is not the unique receptor, the other receptor should have a higher affinity for digitalis than Na^+–K^+-ATPase itself and it should have binding sites for calcium ions. The Na^+–K^+-ATPase could itself fulfil these requirements as it might present more than one binding site for cardiac glycosides, a possibility which is raised by the observation that the capacity of the receptor was different for ouabain and digitoxin [32]; Na^+–K^+-ATPase is inhibited by Ca^{2+} and the action of various glycosides is affected by the calcium content of the perfusion fluid [54, 57].

The search for a Ca^{2+}–digitalis high affinity binding site, sub-unit of the Na^+–K^+-ATPase or constituent of another membrane system, will not only improve our knowledge of the regulatory role of Na^+–K^+-ATPase itself but will also provide a rational approach to the design of new compounds related to digitalis with a better therapeutic index.

REFERENCES

1. Ch. Tamm, *Proc. 1st. Int. Pharmac. Meeting* **3,** 11. Pergamon Press, Oxford (1973).
2. T. Godfraind, *Archs int. Pharmacodyn. Thér.* **206,** 384 (1973).
3. G. A. Langer, *A. Rev. Physiol.* **35,** 55 (1973).
4. H. Reuter, *Circulat. Res.* **34,** 599 (1974).
5. A. M. Katz, *Physiol. Rev.* **50,** 63 (1970).
6. K. S. Lee and W. Klaus, *Pharmac. Rev.* **23,** 193 (1971).
7. A. Wollenberger, *Pharmac. Rev.* **1,** 311 (1949).
8. I. M. Glynn, *Pharmac. Rev.* **16,** 381 (1964).
9. H. J. Schatzmann, *Protoplasma* **63,** 136 (1967).
10. J. C. Skou, *Biochim. biophys. Acta* **23,** 394 (1957).
11. E. T. Dunham and I. M. Glynn, *J. Physiol. Lond.* **156,** 274 (1961).
12. R. Whittam and K. P. Wheeler, *A. Rev. Physiol.* **32,** 21 (1970).
13. P. J. Garrahan and I. M. Glynn, *J. Physiol., Lond.* **192,** 237 (1967).
14. I. M. Glynn, *Br. Med. Bull.* **24,** 165 (1968).
15. R. L. Post, S. Kume, A. K. Sen, T. Tobin and B. Orcutt, *J. gen. Physiol.* **54,** 306 (1969).
16. G. E. Lindenmayer, A. Schwartz and H. K. Thompson, *J. Physiol., Lond.* **236,** 1 (1974).
17. R. Whittam and R. Chipperfield, *Biochim. biophys. Acta* **307,** 563 (1973).
18. J. D. Robinson, *Archs Biochem. Biophys.* **139,** 17 (1970).
19. R. W. Albers and G. J. Koval, *J. biol. Chem.* **248,** 777 (1973).
20. W. M. Hart and E. O. Titus, *J. biol. Chem.* **248,** 1365 (1973).
21. H. Simpkins and L. E. Hokin, *Archs Biochem. Biophys.* **159,** 987 (1973).
22. A. Schwartz, G. E. Lindemayer and J. E. Allen, in *Current Topics in Membranes and Transport*, Vol. 3. Academic Press, New York (1972).
23. Z. M. Bacq, *Experientia* **5,** 207 (1949).
24. L. K. Lane, J. H. Copenhaver, G. E. Lindenmayer and A. Schwartz, *J. biol. Chem.* **248,** 7197 (1973).
25. O. Hansen and J. C. Skou, *Biochim. biophys. Acta* **311,** 51 (1973).
26. E. Erdmann and W. Schoner, *Biochim. biophys. Acta* **307,** 386 (1973); **330,** 302 (1973); **330,** 316 (1973).
27. T. Akera and T. M. Brody, *J. Pharmac. exp. Ther.* **176,** 545 (1971).
28. T. Godfraind and M. Lesne, *Archs int. Pharmacodyn. Thér.* **166,** 195 (1967).
29. K. Kuschinsky, H. Lüllmann and P. A. van Zwieten, *Br. J. Pharmac.* **30,** 317 (1967).
30. S. Dutta, S. Goswami, J. O. Lindower and B. H. Marks, *J. Pharmac. exp. Ther.* **159,** 324 (1968).
31. H. R. Besch, J. C. Allen, G. Glick and A. Schwartz, *J. Pharmac. exp. Ther.* **171,** 1 (1970).
32. T. Godfraind and M. Lesne, *Br. J. Pharmac.* **46,** 488 (1972).
33. T. Godfraind and A. De Pover, *Archs int. Pharmacodyn. Thér.* **201,** 197 (1973).
34. M. Reiter, *Arzneim. -Forsch.* **17,** 1249 (1967).
35. D. Landowne and S. M. Ritchie, *J. Physiol., Lond.* **207,** 529 (1970).
36. P. F. Baker and J. S. Willis, *Nature, Lond.* **226,** 521 (1970).
37. K. Repke and H. J. Portius, *Experientia* **19,** 452 (1963).
38. P. F. Baker, *Progress Biophys. molec Biol.* **24,** 177 (1972).
39. J. Burton and T. Godfraind, *J. Physiol., Lond.* **241,** 287 (1974).
40. A. M. Katz and D. Repke, *Circulat. Res.* **21,** 767 (1967).
41. L. De Meis, *J. biol. Chem.* **246,** 4764 (1971).
42. T. Godfraind, X. Sturbois and N. Verbeke, *Archs int. Physiol. Biochim.* **82,** 748 (1974).
43. E. Carafoli, R. Tiozzo, G. Lugli, F. Grovetti and C. Kratzing, *J. Molec. cell. Cardiol.* **6,** 361 (1974).
44. T. Godfraind and A. Godfraind-De Becker, *Archs int. Pharmacodyn. Thér.* **158,** 453 (1965).
45. T. Godfraind, *Bull. Acad. Roy. Méd. Belg.* **12,** 403 (1972).
46. T. Godfraind, in *Pharmacology and the Future of Man*, Vol. 4, p. 344. Karger, Basel (1973).
47. L. E. Bailey and H. A. J. Sures, *J. Pharmac. exp. Ther.* **178,** 259 (1971).
48. W. G. Nayler, *J. Molec. cell. Cardiol.* **5,** 101 (1973).
49. H. Lüllmann and W. Holland, *J. Pharmac. exp. Thér.* **137,** 186 (1962).
50. G. T. Okita, F. Richardson and B. F. Roth-Schechter, *J. Pharmac. exp. Ther.* **185,** 1 (1973).
51. H. Lüllmann and U. Ravens, *Br. J. Pharmac.* **49,** 377 (1973).
52. T. Peters, R. H. Raben and O. Wassermann, *Eur. J. Pharmac.* **26,** 166 (1974).
53. E. Heinen and E. Noack, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmac.* **275,** 359 (1972).
54. R. G. Alken, V. Fricke and W. Klaus, *Eur. J. Pharmac.* **26,** 331 (1974).
55. T. Tobin, T. Akera, D. Ku and M. C. Lu, *Molec. Pharmac.* **9,** 676 (1973).
56. T. Akera, S. I. Baskin, T. Tobin and T. M. Brody, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmac.* **277,** 151 (1973).
57. T. Tobin, T. Akera, S. I. Baskin and T. M. Brody, *Molec. Pharmac.* **9,** 336 (1973).
58. P. Muller, *Circulat. Res.* **17,** 46 (1965).
59. W. C. Appel and F. F. Vincenzi, *J. Pharmac. exp. Ther.* **187,** 112 (1973).
60. N. O. Kim, L. E. Bailey and P. E. Dresel, *J. Pharmac. exp. Ther.* **181,** 377 (1972).
61. W. D. M. Paton and J. P. Payne, *Pharmacological Principles and Practice*, p. 185. Churchill, London (1968).
62. R. Niedergerke and H. C. Lüttgau, *Nature* **179,** 1066 (1957).
63. P. C. Caldwell and R. D. Keynes, *J. Physiol., Lond.* **148,** 8 (1959).