Biochemical Pharmacology, Vol. 33, No. 22, pp. 3649–3654, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

SPECIES VARIATION IN THE OUABAIN SENSITIVITY OF CARDIAC Na^+/K^+-ATPase

A POSSIBLE ROLE FOR MEMBRANE LIPIDS

MAHINDA Y. ABEYWARDENA*, EDWARD J. MCMURCHIE, GORDON R. RUSSELL and JOHN S. CHARNOCK

Division of Human Nutrition, Commonwealth Scientific and Industrial Research Organization, Glenthorne Laboratory, O'Halloran Hill, South Australia 5158

(*Received* 12 *March* 1984; *accepted* 25 *June* 1984)

Abstract—The role of membrane lipid composition on the modulation of ouabain sensitivity of cardiac Na^+/K^+-ATPase has been studied *in vitro* using several animal species. The animals can be grouped as ouabain-sensitive and ouabain-insensitive species. Ouabain-sensitive species (I_{50}; 0.5–2.2 μM) include sheep, marmoset, pig and the guinea pig, whilst rat and mouse form the ouabain-insensitive group (I_{50}; 100–105 μM). Although no species variation in the distribution of major phospholipid classes was observed, significant differences were apparent in the proportions of certain saturated and unsaturated phospholipid fatty acids. Thus, there was a marked increase in the relative proportion of docosahexaenoic (22:6, ω-3) acid in the Na^+/K^+-ATPase preparations from the rat and mouse compared to ouabain-sensitive species. Despite these differences, all animals had similar proportions of total saturated (ΣSAT) and total unsaturated (ΣUnsat) fatty acids. On the other hand, a good correlation between the unsaturation index of membrane lipids and I_{50} value for ouabain was observed. It is proposed that acyl chain characteristics (unsaturation and/or chain length) rather than the head group of the phospholipid molecule play a major role in the modulation of Na^+/K^+-ATPase to inhibition by ouabain.

The cardiotonic action of digitalis glycosides and their use in treating the failing heart was first reported by Withering in 1783 [1]. Yet, two centuries later, the exact mechanism of positive inotropic action of cardiac glycosides is not completely elucidated [2]. However, it is generally believed that the pharmacological response of heart to cardiac glycosides is a consequence of interaction of these drugs with cardiac Na^+/K^+-ATPase [3–8], although dissociation of the positive inotropic effect from inhibition of Na^+/K^+-ATPase has occasionally been reported [9–12].

It is known that Na^+/K^+-ATPase preparations from different species display varying degrees of "sensitivity" to cardiac glycoside inhibition [4, 13]. For example, Na^+/K^+-ATPase from human, cat and dog are said to be highly sensitive, rabbit and guinea pig are moderately sensitive, whereas the enzyme preparation from rat heart is reported to be rather insensitive to inhibition by digitalis glycosides [14–18]. However, detailed analysis of the ATPase protein has revealed that it is remarkably constant between species [19, 20]. In addition, the cardiac glycoside receptor densities in the membrane appear to be quite similar between digitalis-sensitive and digitalis-insensitive species [21–23]. Thus, no clear explanation has been found for these apparent differences in "sensitivity", although several suggestions have been made [14, 17, 24]. Such observations should focus attention on the possible role of membrane lipids in modulating the ouabain sensitivity of Na^+/K^+-ATPase. Indeed, lipid modulation of ouabain sensitivity of neuronal Na^+/K^+-ATPase has been demonstrated recently [25].

In the present study, we have further examined the role of membrane lipids in modulating the ouabain sensitivity of cardiac Na^+/K^+-ATPase using preparations from different species. We report that the "sensitivity" of Na^+/K^+-ATPase to inhibition by ouabain appears to be influenced by the acyl chain characteristics (chain length, unsaturation) of the surrounding phospholipids.

A preliminary account of this work has been presented to the Australian Biochemical Society [26].

MATERIALS AND METHODS

Methods. The animals used in the present study include rat, sheep, pig, guinea pig, mouse and the common marmoset monkey (*Callithrix jacchus jacchus*). The ventricle tissue, dissected free of connective tissue and stored in 60% glycerol (in 20 mM Tris–1 mM EDTA, pH 7.6: buffer I) at −80°, was thawed and rinsed several times with ice-cold buffer I. The tissue (approx. 5–10 g) was chopped and disrupted in a medium containing 250 mM sucrose–30 mM L-histidine and 20 mM Tris, pH 6.8, using a Polytron tissue disintegrator (PT35; kinematica GmbH-Switzerland) at a setting of 3.5. Three separate bursts of 10, 10 and 15 sec were employed to disrupt the tissue. The resultant brei was centrifuged at 1000 *g* for 15 min and the pellets were resuspended (25 ml/g tissue wet weight) in 1 M KCl (in buffer I, pH 7.6) and extracted with an equal volume of 2 M

* To whom all correspondence should be addressed.

NaI (in buffer, I, pH 8.0) at 0° for 60 min. For the preparation of pig enzyme 4 M NaI was used. At the end of the extraction period the mix was centrifuged at 27,000 *g* for 20 min and the pellet was resuspended in buffer I and filtered through cheesecloth before centrifugation at the above speed. The resulting pellet was resuspended in 250 mM sucrose–20 mM Tris–1 mM EDTA, pH 7.6 (buffer II) to yield a protein concentration of approximately 5 mg/ml and stored frozen overnight at −80°.

After thawing, the NaI-treated pellet was further extracted for 30 min at 30° with 0.05% (w/v) deoxycholate at a detergent/protein ratio of 1:1 (w/w in the presence of KCl (250 mM) and Na_2-ATP (5 mM)). At the end of extraction, this membrane/detergent suspension was centrifuged at 10,000 *g* for 20 min and the resulting supernatant was further centrifuged at 48,000 *g* for 60 min to yield a membrane preparation enriched in Na^+/K^+-ATPase activity. The pellets were washed twice by resuspension and centrifugation in buffer I prior to taking up in buffer II to give a protein concentration of approximately 1 mg/ml, and used for enzyme assays and lipid analysis. Protein was determined by the method of Peterson [27], using fat-free bovine serum albumin as standard.

Assay of Na^+/K^+*-ATPase.* The activity of enzyme in the absence and presence of various concentrations of ouabain was determined using the coupled assay method described elsewhere [25, 28]. The assay mix [25, 29], with or without ouabain, containing 10–20 μg enzyme protein, was incubated at 37°. After temperature equilibration, the reaction was initiated by the addition of Tris–ATP to a final concentration of 1.5 mM. The final assay volume was 520 μl. The decrease in absorbance was monitored continuously at 340 nm, using a Gilford spectrophotometer (model 250). The percent ouabain sensitivity of the enzyme preparations ranged from 88% (rat) to 99% (pig).

Lipid extraction and analysis. Membrane enzyme preparations (in distilled water) were extracted according to the method of Bligh and Dyer [30] with chloroform:methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene. Phospholipids were separated from other lipids by chromatography on Kieselgel 60 H plates [31]. A portion of the phospholipid fraction was heated at 70° for 30 min in a sealed glass tube containing borontrifluoride, methanol and H_2SO_4 to prepare the total phospholipid fatty acid methyl esters [31]. The methyl esters were analysed by gas liquid chromatography. Columns were packed with 5% SP-2310 on 100/120 chromosorb WAW (Supelco Inc., Bellafonte, PA). The remaining phospholipid fraction was used to determine the distribution of major phospholipid classes by the method of Skipski and Barclay [32]. Phosphate levels were estimated by the malachite green method as described by Bowyer and King [33].

Chemicals. Ouabain octahydrate, pyruvate kinase (Type II), lactate dehydrogenase, NADH (cyclohexylamine salt) phospho-*enol*-pyruvate (trimonocyclohexylammonium salt) and deoxycholate (sodium salt) were all purchased from Sigma Chemical Co., St. Louis, MO. Sodium iodide (AR) was supplied by BDH Chemicals (U.K.). Tris–ATP was prepared by ion exchange chromatography [25]. All other chemicals were of analytical grade purity.

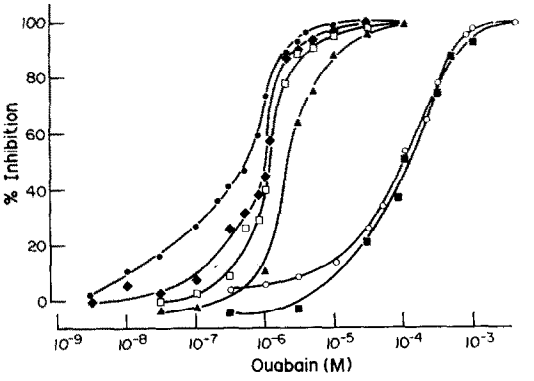

Fig. 1. Mean dose–response curves for ouabain inhibition of cardiac Na^+/K^+-ATPase from different species. Enzyme preparations were assayed at 37° in the presence of 80 mM Na^+, 20 mM K^+, 2 mM Mg^{2+}, 1.5 mM ATP and varying concentrations of ouabain as described in the text. For each species, data shown are the mean values of four separate experiments. Error bars have been omitted for clarity. (●) sheep; (◆) marmoset; (□) pig; (▲) guinea pig; (○) rat; (■) mouse.

RESULTS

The mean dose–response curves for ouabain inhibition of cardiac Na^+/K^+-ATPase preparations from different animal species are shown in Fig. 1. The specific activity and the concentration of ouabain which caused half-maximal inhibition of enzyme activity (I_{50} value) of each enzyme preparation are summarized in Table 1. It is evident that the potency of ouabain inhibition of cardiac Na^+/K^+-ATPase differs considerably among the animal species investigated, as mean I_{50} values ranged from 0.5 μM for sheep to 105 μM for the enzyme isolated from mouse heart. Therefore, the order (high → low) of sensitivity to ouabain inhibition is sheep > marmoset ≥ pig > guinea pig >>> rat ≥ mouse. A fourfold increase in the I_{50} value, compared to that of sheep, was observed for the guinea pig enzyme, as 2.2 μM ouabain was required to produce 50% inhibition of enzyme activity. In comparison to the other four

Table 1. Specific activity and sensitivity to inhibition by ouabain of cardiac Na^+/K^+-ATPase preparations from various species

Species	I_{50} (μM)	Spec. act.*
Sheep	0.5 ± 0.1	11.9 ± 2.3
Marmoset	1.1 ± 0.4	12.7 ± 2.4
Pig	1.3 ± 0.5	19.8 ± 1.8
Guinea pig	2.2 ± 0.8	10.8 ± 2.8
Rat	100 ± 12.0	13.1 ± 1.1
Mouse	105 ± 11.1	9.9 ± 1.4

All values are mean ± S.E.M. (N = 4).

* Specific activity—μmol ATP hydrolyzed/mg protein per hr at 37°.

Table 2. Phospholipid class distribution of cardiac Na^+/K^+-ATPase preparations from different animal species

Lipid class*	Sheep	Marmoset	Pig	Guinea pig	Rat	Mouse
PC	50.5 ± 1.7	48.9 ± 1.4	49.0 ± 1.6	48.0 ± 0.9	48.1 ± 1.1	49.8 ± 0.8
PE	33.4 ± 2.1	34.6 ± 0.8	36.2 ± 1.2	36.9 ± 0.9	36.2 ± 1.3	33.2 ± 0.8
DPG	8.1 ± 0.6	9.4 ± 1.3	8.2 ± 1.4	7.0 ± 0.4	8.8 ± 0.6	8.9 ± 0.9
SM + PS + PI	7.3 ± 0.9	7.8 ± 1.1	8.3 ± 0.9	8.2 ± 0.6	6.8 ± 1.0	7.0 ± 0.7

Values given are mol% of total phospholipids (mean ± S.E.M.; N = 4).
* Phosphatidylcholine (PC); phosphatidylethanolamine (PE); diphosphatidylglycerol (DPG); sphingomyelin (SM); phosphatidylserine (PS); phosphatidylinositol (PI).

species, marked increases in the I_{50} value were apparent when both rat and mouse Na^+/K^+-ATPase were examined. Thus, enzyme preparations from these two species had mean I_{50} values of 100 and 105 μM, respectively, which were almost 200-fold greater than that observed for the sheep. It is also clear that enzyme preparations from all animal species had appreciable, yet similar, levels of specific activities (Table 1).

The distribution of phospholipid classes of cardiac Na^+/K^+-ATPase from different animal species is shown in Table 2. It is clear that together phosphatidylcholine and phosphatidylethanolamine account for more than 80% of the total phospholipid present in these membrane enzyme preparations. It is also evident that the proportions of these two major phospholipids were similar between the animal species studied. The contribution from diphosphatidylglycerol (cardiolipin) was about 8% in enzyme preparations from all animal species and sphingomyelin, phosphatidylserine and phosphatidylinositol accounted for the remaining 7–9% of the total phospholipids.

In contrast to the distribution of phospholipid classes, dramatic differences were found in the relative proportions of certain phospholipid fatty acids from these membrane preparations. Table 3 shows the fatty acid composition of total phospholipids of various cardiac Na^+/K^+-ATPase preparations. It can be seen that whilst the relative proportion of palmitic (16:0) acid was similar, some species variation was observed in the proportion of stearic (18:0) acid, the other major saturated fatty acid in these membranes. Pig and sheep membranes had only 14–16% of total fatty acids as stearic acid, compared to 19–23% in the case of rat and mouse. On the other hand, compared to other animals, both sheep and pig contained relatively high proportions of the dimethylacetal (DMA) derivatives—with the total 16:0 and 18:0 DMA proportions being 8.9% (sheep) and 10.9% (pig) of the total phospholipid fatty acids. Dimethylacetal compounds are produced from alkenylethers of plasmalogen phospholipids during the acid-catalysed methylation process [34]. All animals contained a relatively small (less than 1.4%) proportion of long chain saturated lignoceric (24:0) acid.

Dramatic differences in the distribution of certain

Table 3. Major phospholipid fatty acids of cardiac membrane preparations enriched in Na^+/K^+-ATPase from different species

Fatty acid (% w/w)	Sheep	Marmoset	Pig	Guinea pig	Rat	Mouse	LSD*
DMA 16:0	7.4	1.3	10.2	2.8	1.4	1.8	2.7
16:0	10.8	10.6	10.9	10.2	11.2	13.3	3.8
DMA 18:0	1.5	3.2	0.7	3.9	0.7	0.7	2.3
18:0	16.1	18.5	14.3	18.4	22.6	19.4	1.3
18:1 (ω-9)	13.3	12.4	12.6	8.2	8.3	8.6	3.0
18:2 (ω-6)	27.8	23.0	27.4	30.2	23.0	13.4	6.3
20:4 (ω-6)	14.6	19.2	18.2	19.0	15.9	5.4	5.7
22.4 (ω-6)	0.8	0.5	0.7	0.3	0.3	0.2	0.4
24:0	0.3	1.5	0.3	0.5	0.3	0.4	0.5
22:5 (ω-3)	2.2	2.1	1.6	1.4	1.0	1.0	0.5
22:6 (ω-3)	1.4	4.4	0.7	1.0	14.8	33.4	3.2
Σ Sat.	36.1	35.1	36.4	35.8	36.2	35.6	
Σ Unsat.	60.1	61.6	61.2	60.1	63.3	62.0	
Σ ω-6	43.2	42.7	46.3	49.5	39.2	19.0	
Σ ω-3	3.6	6.5	2.3	2.4	15.8	34.4	
ω-6/ω-3	12.0	6.6	20.1	20.6	2.5	0.5	

All values are the mean of four determinations.
* LSD—Least significance difference calculated from error estimates of analysis of variance (51). Mean values within a row differing by more than the LSD are significant at the 1% level.
DMA—dimethylacetal derivative; ω number designates the position of the first double bond from the terminal methyl group.

unsaturated fatty acids were observed between enzyme preparations from different animal species (Table 3). For instance, the proportion of monounsaturated oleic (18:1) acid was higher in the sheep, marmoset monkey and pig compared to preparations from guinea pig, rat and mouse. All animals except mouse contained appreciably high but similar proportions of ω-6 unsaturated linoleic (18:2, ω-6) and arachidonic (20:4, ω-6) acids. In the mouse, these two major fatty acids of the ω-6 family accounted for only 19% of total fatty acids compared to 38–50% in all other species. However, the reduced levels of ω-6 fatty acids in the mouse was compensated for by a marked increase in the proportion of ω-3 unsaturated docosahexaenoic (22:6) acid. For example, this particular long chain polyunsaturated fatty acid (PUFA) alone represented over 30% of the total fatty acids of Na^+/K^+-ATPase from mouse heart. Similarly, enzyme preparations from the rat also contained a relatively high proportion of 22:6, compared to ouabain-"sensitive" (Fig. 1 and Table 1) species—sheep, pig, marmoset and guinea pig, all of which had relatively low levels (less than 4%) of this long chain PUFA. Enzyme preparations from all animal species contained only 1–2% docosapentaenoic (22:5) acid, the only other ω-3 series fatty acid detected in these membranes. As a result of the elevated levels of the ω-3 fatty acid 22:6, both rat and mouse displayed a relatively low ω-6/ω-3 ratio compared to other animal species (Table 3). However, it was of interest to note that, in spite of the differences in the relative proportions of certain fatty acids, the overall proportions of both total saturated (ΣSat) and total unsaturated (ΣUnsat) fatty acids were similar between all animal species.

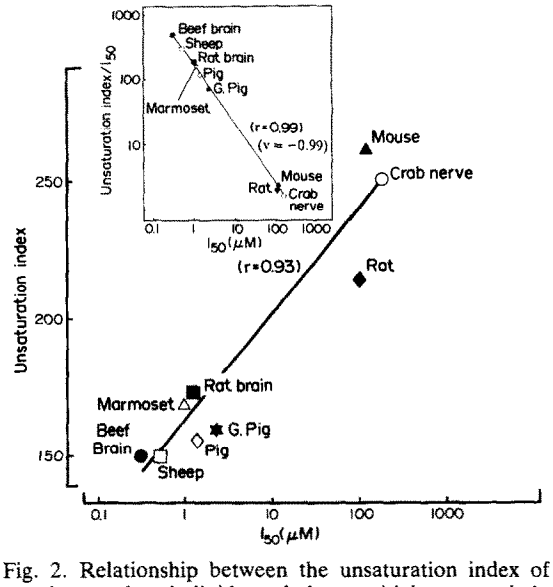

Fig. 2. Relationship between the unsaturation index of membrane phospholipids and the sensitivity to ouabain inhibition of cardiac Na^+/K^+-ATPase from various species. The unsaturation index for each species is Σ[(a)(b)] and was calculated from the data given in Table 3 where (a) is the relative proportion of each unsaturated fatty acid and (b) is the number of double bonds for that particular fatty acid. The insert shows the same data when plotted as unsaturation index/I_{50} vs I_{50}.

The relationship between the I_{50} value for ouabain sensitivity of cardiac Na^+/K^+-ATPase of each animal species and the unsaturation index (calculated from the mono- and polyunsaturated fatty acids) of the corresponding membrane preparation is illustrated in Fig. 2. The values for beef brain and crab nerve Na^+/K^+-ATPase preparations are from our previous studies [25, 35]. It can be seen that there appears to be a good correlation (r = 0.93) between unsaturation index on the one hand and I_{50} value for ouabain inhibition on the other for Na^+/K^+-ATPase isolated from neural and cardiac tissue of a variety of species. In addition, variation in the ouabain sensitivity of membrane Na^+/K^+-ATPase from different tissues of the same species (i.e. rat brain and heart) appears to correlate well with their respective unsaturation indices. It is of great interest to note that a better correlation (r = −0.99) between membrane unsaturation and ouabain sensitivity was seen when unsaturation index/I_{50} was plotted against the I_{50} value (Fig. 2 insert), indicating a complex interaction between membrane lipids and ouabain sensitivity.

DISCUSSION

In the present study considerable species variation was observed in the sensitivity of cardiac Na^+/K^+-ATPase to inhibition by ouabain. The animals investigated can clearly be separated into two groups—as ouabain "sensitive" and ouabain "insensitive" species. Sheep, marmoset monkey, pig and guinea pig were the ouabain-sensitive species whilst rat and mouse formed the "insensitive" group. The I_{50} values reported in the present study are comparable with those reported by other workers [13–16]. However, to our knowledge, no literature is available on the ouabain inhibition of both mouse and marmoset monkey Na^+/K^+-ATPase.

The significantly high I_{50} values for ouabain inhibition of rat and mouse cardiac Na^+/K^+-ATPase compared to those of the sensitive group cannot be accounted for by differences in the distribution of phospholipid classes (Table 2). Several workers have reported that certain phospholipids, particularly the negatively charged phosphatidylserine and phosphatidylinositol, are important in determining the activity of Na^+/K^+-ATPase [36–40]. However, the effect of these phospholipids on the ouabain receptor properties of Na^+/K^+-ATPase has not been reported. In addition, the apparent insensitivity of crab nerve Na^+/K^+-ATPase to ouabain inhibition does not appear to be due to a head group effect, but is thought to be influenced by the acyl chain properties of the surrounding phospholipids [25, 35].

It was of interest to note that membrane preparations enriched in Na^+/K^+-ATPase from all animal species investigated had similar proportions of both total saturated (ΣSat) and total unsaturated (ΣUnsat) fatty acids, in spite of the significant differences in the proportions of certain individual fatty acids (Table 3). This may perhaps indicate some form of regulatory or homeostatic mechanism, as this membrane system, namely the sarcolemma, hosts a number of lipid-dependent processes which are of key importance to normal cardiac function [41, 42].

The insensitivity of rat heart Na^+/K^+-ATPase has

been attributed by Allan and Schwartz [14] to an unstable ouabain–enzyme complex and also to binding of ouabain to sites unrelated to enzyme inhibition. According to Erdmann *et al.* [21] the suggestion of Tobin and co-workers [17, 18], that species difference in ouabain insensitivity may be caused by a rapid dissociation of the drug from its receptor, does not give a comprehensive explanation. Several other workers have reported the presence of low and high affinity ouabain binding sites [43–47]. Occupation of high affinity sites has been correlated to positive inotropic effect without inhibition of Na^+/K^+-ATPase activity or sodium pump activity, at least in the rat heart [21]. On the other hand, occupation of low affinity receptors is thought to lead to sodium pump inhibition [48]. As only low affinity receptors were found in cat heart—a digitalis-sensitive species—Erdmann, Philipp and Scholz [47] speculated that digitalis-sensitive species might have low affinity receptors, whereas the digitalis-insensitive animals may contain both types of binding sites. However, it can be argued that these reported different binding sites for ouabain are in fact two different conformations of the same receptor or binding site. Moreover, it is tempting to suggest in the light of the present data that these conformation changes of the enzyme (receptor) protein are influenced by the phospholipid acyl chain properties, particularly the chain length and the level of unsaturation of surrounding lipids. In this context it should be remembered that both rat and mouse Na^+/K^+-ATPase preparations contained considerably higher proportions of 22:6 (ω-3) than those from "ouabain-sensitive" animal species. However, this alone does not give a comprehensive explanation for the variation in sensitivity, as Na^+/K^+-ATPase preparations from neural tissues, such as beef or rat brain, also contain relatively high proportions of 22:6 (approx. 12–18%), yet are rather sensitive to ouabain inhibition [25, 49]. In contrast, a good correlation between the overall unsaturation index of membrane phospholipids and sensitivity of Na^+/K^+-ATPase to inhibition by ouabain was observed for both cardiac and neural enzyme preparations (Fig. 2). Therefore, it seems likely that differences in membrane physical properties may play a role, at least in part, in the sensitivity of cardiac Na^+/K^+-ATPase to inhibition by ouabain. In accord with the findings of the present study, Schwalb *et al.* [50] recently reported that structural components of the membrane other than the primary structure of the enzyme protein influence the ouabain sensitivity of Na^+/K^+-ATPase.

It should be pointed out that the present study does not exclude the possibility that differences in the structure of the Na^+/K^+-ATPase protein may also be in part responsible for the apparent species variation in ouabain-sensitivity. However, the role of lipids of the membrane matrix in modulating the interaction of cardiac glycosides such as ouabain with its Na^+/K^+-ATPase receptor appears to be a crucial one.

REFERENCES

1. W. Withering, in *Medical Classics* Vol. II, (Ed. E. M. Kelly), pp. 305–443. Williams & Wilkins, Baltimore (1937).
2. S. Noble, *Cardiovasc. Res.* **14**, 495 (1980).
3. K. R. H. Repke, in *New Aspects of Cardiac Glycosides*, (Vol. III, (Ed. W. Wilbrandt), pp. 47–73, *Proc. 1st Int. Pharmacological Meeting*, Stockholm, 1961, Pergamon, London (1963).
4. A. Schwartz, G. E. Lindenmayer and J. C. Allen, *Pharmac. Rev.* **27**, 3 (1975).
5. A. Schwartz, *Circ. Res.* **39**, 2 (1976).
6. T. Akera and V. J. K. Cheng, *Biochim. biophys. Acta.* **470**, 412 (1977).
7. G. A. Langer, *Fedn. Proc. Fedn. Am. Soc. exp. Biol.* **36**, 2231 (1977).
8. T. Akera and T. M. Brody. *Pharmac. Rev.* **29**, 187 (1978).
9. G. T. Okita, F. Richardson and B. F. Roth-Schechter, *J. Pharmac. exp. Ther.* **185**, 1 (1973).
10. G. T. Okita and R. E. Ten Eick, *Ann. N. J. Acad. Sci.* **242**, 658 (1974).
11. T. Peters, R. H. Raben and O. Wasserman. *Eur. J. Pharmac.* **26**, 166 (1974).
12. H. M. Rhee, S. Dutta and B. H. Marks, *Eur. J. Pharmac.* **37**, 141 (1976).
13. J. S. Charnock, W. F. Dryden and P. A. Lauzon, *Comp. Biochem. Physiol.* **66C**, 153 (1980).
14. J. C. Allen and A. Schwartz, *J. Pharmac. exp. Ther.* **168**, 42 (1969).
15. T. Akera, F. S. Larsen and T. M. Brody, *J. Pharmac. exp. Ther.* **170**, 17 (1969).
16. T. Tobin and T. M. Brody, *Biochem. Pharmac.* **21**, 1553 (1972).
17. T. Tobin, R. Henderson and A. K. Sen. *Biochim. biophys. Acta* **274**, 551 (1972).
18. T. Akera, S. I. Baskin, T. Tobin and T. M. Brody, *Naunyn-Schmiedeberg's Arch. Pharmac.* **277**, 151 (1973).
19. G. L. Peterson and L. E. Hokin, *J. biol. Chem.* **256**, 3751 (1981).
20. L. E. Hokin, *Ann. N.Y. Acad. Sci.* **242**, 12 (1974).
21. E. Erdmann, G. Philipp and H. Scholz, *Biochem. Pharmac.* **29**, 3219 (1980).
22. L. H. Micheal, A. Schwartz and E. T. Wallick, *Molec. Pharmac.* **16**, 135 (1979).
23. D. McCall, *Am. J. Physiol.* **236**, C87 (1979).
24. A. de Pover and T. Godfraind, *Biochem. Pharmac.* **28**, 3051 (1979).
25 M. Y. Abeywardena and J. S. Charnock, *Biochim. biophys. Acta* **729**, 75 (1983).
26. M. Y. Abeywardena, E. J. McMurchie, J. S. Charnock and G. R. Russell, *Proc. Aust. Biochem. Soc.* **15**, 86 (1983).
27. G. L. Peterson, *Analyt. Biochem.* **83**, 346 (1977).
28. W. Schoner, C. Von Ilberg, R. Kramer and W. Seubert, *Eur. J. Biochem.* **1**, 334 (1967).
29. J. S. Charnock, L. P. Simonson and A. F. Almeida, *Biochim. biophys. Acta* **465**, 77 (1977).
30. E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.* **37**, 911 (1959).
31. J. S. Charnock, W. F. Dryden, E. J. McMurchie, M. Y. Abeywardena and G. R. Russell, *Comp. Biochem. Physiol.* **75B**, 47 (1983).
32. V. P. Skipski and M. Barclay, in *Methods in Enzymology*, Vol. XIV, (Ed. J. Lowenstein), pp. 530–598, Academic Press, London (1969).
33. D. E. Bowyer and J. P. King, *J. Chromatogr.* **143**, 473 (1977).
34. J. K. G. Kramer, E. R. Farnworth, B. K. Thompson, A. H. Corner and H. L. Trenholm, *Lipids* **17**, 372 (1982).
35. J. S. Charnock and L. P. Simonson, *Comp. Biochem. Physiol.* **58B**, 381 (1977).
36. H. K. Kimelberg and D. Papahadjopoulos, *Biochim. biophys. Acta* **282**, 277 (1972).
37. J. G. Mandersloot, B. Roelofsen and J. de Gier, *Biochim. biophys. Acta* **508**, 478 (1978).

38. B. Roelofsen and L. L. M. Van Deenen, *Eur. J. Biochem.* **40**, 245 (1973).
39. H. K. Kimelberg and D. Papahadjopoulos, *J. biol. Chem.* **249**, 1071 (1974).
40. B. Roelofsen, *Life Sci.* **29**, 2235 (1981).
41. N. S. Dhalla, A. Ziegelhoffer and J. A. Harrow, *Can. J. Physiol. Pharmac.* **55**, 1211 (1977).
42. A. M. Katz and F. C. Messineo, *Circ. Res.* **48**, 1 (1981).
43. O. Hansen, *Biochim. biophys. Acta* **443**, 383 (1976).
44. W. Schoner, H. Pauls and R. Patzelt-Wenezler, in *Myocardial Failure* (Eds. G. Riecker, A. Weber and J. Goodwin), pp. 104–119, Springer-Verlag, Berlin (1977).
45. E. Erdmann, W. Krawietz and P. Presok, in *Myocardial Failure* (Eds. G. Riecker, A. Weber and J. Goodwin), p. 120 Springer, Berlin (1977).
46. J. Ghysel-Burton and T. Godfraind, *Br. J. Pharmac.* **66**, 175 (1979).
47. E. Erdmann, G. Philipp and H. Scholz, in *Cell Membrane in Function and Dysfunction of Vascular Tissue* (Eds. T. Godfraind and P. Meyers), pp. 76–83. Elsevier/North Holland, Amsterdam (1981).
48. T. Godfraind, *Biochem. Pharmac.* **24**, 823 (1975).
49. M. Y. Abeywardena and G. R. Russell, unpublished data.
50. H. Schwalb, Y. Dickstein and M. Heller, *Biochim. biophys. Acta* **689**, 241 (1982).
51. D. Colquhoun, in *Lectures on Biostatics*, pp. 210–213. Clarendon Press, Oxford (1971).