Br. J. Pharmac. (1981), 73, 81–86

ACCUMULATION OF [^{3}H]-OUABAIN IN FUNCTIONALLY DIFFERENT CANINE CARDIAC TISSUES: DIFFERENTIAL Rb^+ UPTAKE

H.M. RHEE[1]

Department of Pharmacology, Medical College of Ohio, C.S. 10008, Toledo, Ohio 43699, U.S.A.

1 In order to understand tissue-specific differences in sensitivity to ouabain, the distribution of [^{3}H]-ouabain was investigated in seven functionally different, canine cardiac tissues.

2 Specialized cardiac tissues (e.g., pacemaker or conducting tissues) accumulate much less ouabain than do the contractile tissues such as the left ventricule or the papillary muscle.

3 This study confirms a greater Rb^+ uptake in the Purkinje fibre than in the contractile tissue.

4 The results disprove the hypothesis that a high accumulation of ouabain in the Purkinje fibre is responsible for the well-known sensitivity to ouabain in this tissue.

Introduction

Functionally different cardiac tissues (e.g., impulse generating or conducting specialized tissues and mechanically contracting ventricular muscle) have different electrochemical and physiological properties (Cranefield, 1975; Sherf & James, 1979). The intracellular ionic distribution of the sino-atrial node or other specialized cardiac conducting fibres differs from the ionic concentrations of contractile ventricular muscle (Polimeni, 1974; Vick, Chang, Nichols, Hazelwood & Harvey, 1973). Electrophysiological sensitivity to cardiac steroids such as ouabain in the canine Purkinje fibre exceeds the sensitivity to ouabain in the ventricle (Vassalle, Karis & Hoffman, 1962). For instance, electrical inexcitability induced by ouabain in canine Purkinje fibre occurred significantly earlier than that in the ventricle from the same animal. Strophanthin K decreased conduction velocity in canine Purkinje fibres at a concentration which did not significantly alter the parameter in the ventricular muscle (Moe & Mendez, 1951). It is not known whether such differential sensitivity to cardiac steroids is related to the functional role of the specific tissues. Furthermore the pharmacological basis of the differential sensitivity to ouabain among cardiac tissues has not been established. Therefore, the purpose of this investigation was to correlate the magnitude of the active transport of Rb^+ and the amount of [^{3}H]-ouabain accumulated in the tissue in several functionally different cardiac tissues.

[1] Present address: Dept. of Pharmacology, Oral Roberts University, 7777 South Lewis, Tulsa, Oklahoma 74171, U.S.A.

Methods

Experiments were conducted with healthy mongrel dogs of either sex, and of 15 to 25 kg body weight. A right thoracotomy or a midline sternotomy was performed under pentobarbitone (30 mg/kg i.v.) anaesthesia and the heart was quickly excised and left beating in ice-chilled saline to wash out the blood in the chambers. In each heart, seven different cardiac tissues were identified and removed for the study of cation active transport and for the determination of tissue ouabain content.

Tissue identification

The sino-atrial node (SAN) was identified by the sinus nodal artery, the first branch of the right coronary artery. The SAN lies along the sulcus terminalis at the junction of the superior vena cava and the right atrium (James, 1962) in dogs. Its posterior position is at the junction of the atrium with the sinus intercavacum. The atrio-ventricular node (AVN) is in the lower interatrial septum below the fossa ovalis, anterior to the ostium of the coronary sinus. The AVN is identified by a white thin compact sheet of muscle fibres beneath the right atrial endocardium and directly above the insertions of the tricuspid valve. Since it is impossible to dissect the SAN or the AVN in pure form, in the initial study the contamination of other tissues was routinely examined. Each nodal tissue was stained by a conventional method (Clark, 1973) with hematoxylin and eosin and cut into 20 to 40 tissue blocks. Randomly selected 10 to 20 tissue blocks (50% of total tissues) were examined

0007–1188/81/050081–006 $01.00

© Macmillan Publishers Ltd 1981

under a light microscope. The number of tissue blocks containing non-nodal tissue was expressed as a percentage of the total tissue blocks as an index of contamination.

Strands of free-running Purkinje fibre (false-tendon) from both the ventricles were easily identified. The papillary muscle of the right ventricle and a piece of the right atrium and the ascending aorta were also removed. A piece of the left ventricle was used after removal of the epicardial and the endocardial layers. The entire procedure was carried out at 4°C, and usually took 5 to 7 min after removal of the heart. Upon isolation of these tissues each was immediately incubated in K^+-free cold Krebs-Henseleit (K-H) solution (Winegrad & Shanes, 1962), pre-equilibrated with 95% O_2 and 5% CO_2 (pH 7.4) at 4°C for 30 min to load Na^+. Tissue slices (0.5 mm thickness) were prepared with a McIlwain tissue chopper (Brinkman Instrument, Westbury, N.Y.) after a brief trimming of fat and connective tissues.

Determination of $^{86}Rb^+$ transport

The modified method of Ku, Akera, Pew & Brody (1974) originally described by Bernstein & Israel (1970) was used to determine the active transport of Rb^+. The tissue blocks, weighing 3 to 15 mg each, were incubated in a final 10 ml of K^+-free K-H solution containing 2mM RbCl and a trace amount of $^{86}Rb^+$ (265 mCi/mmol, New England Nuclear Corp., Boston, Mass.) at 30°C, and the tissue slices were then rinsed three times in 100 ml of the K^+-free solution containing 2 mM RbCl at 0°C. The tissues were gently blotted and counted immediately for $^{86}Rb^+$ in 2 ml of K-H solution according to the principle of Cerenkov radiation (Hougen and Smith, 1978). Based on tissue-wet weight, Rb^+ uptake was expressed in either tissue to medium ratio or in nmol Rb^+ per mg tissue at given time intervals. Different tissue slices from the same animal were also incubated separately in the presence of 0.1 mM ouabain for the assessment of non-specific $^{86}Rb^+$ accumulation. The difference between this non-specific accumulation and the total Rb^+ uptake was considered as ouabain-sensitive, active transport of Rb^+. Experiments were also carried out at 0°C or in the presence of 1 mM iodoacetic acid to test whether Rb^+ uptake meets the criteria of active transport.

[^3H]-ouabain accumulation studies in vivo

Saline was infused continuously in control dogs at the rate of 0.05 ml min^{-1} kg^{-1} for 5 h. In another group of animals ouabain was infused at the speed of 2.6 μg kg^{-1} h^{-1} after a loading dose of ouabain (20 μg/kg) with sufficient [^{3}H]-ouabain for 5 h as reported previously (Rhee *et al.*, 1976). This specific dose of ouabain is referred to as the 'therapeutic dose' because it did not induce any undesirable toxic effects but only the significant positive inotropic action of ouabain. Doubling the loading and infusion doses of ouabain, referred to as the 'toxic dose' produced persistent cardiac arrhythmias (Rhee, Dutta & Marks, 1976). Seven different cardiac tissues were quickly isolated as described previously after a 5 h control period or drug intervention, and the [^{3}H]-ouabain content was determined as described by Dutta, Goswami, Datta, Lindower & Marks (1968). Ouabain accumulation was expressed either in pmol of ouabain per g of tissues (wet weight) or in pmol of ouabain per mg protein after the determination of protein content in tissue homogenate by the method of Lowry, Rosenbrough, Farr & Randall (1951).

Summarized data are represented by the arithmetic mean and dispersion of the data are expressed as standard error of the mean (s.e. mean). Statistical

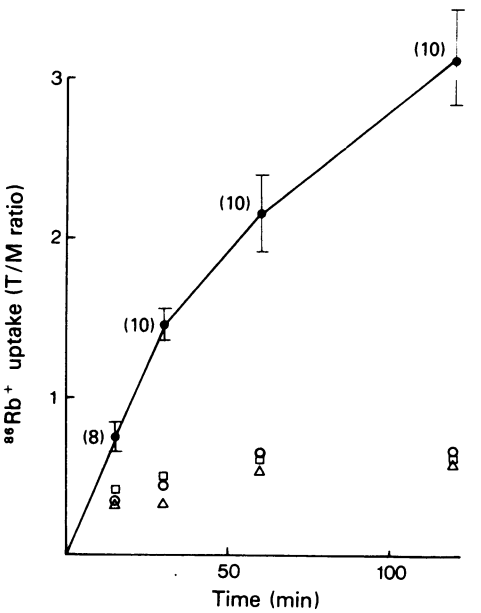

Figure 1 Characteristics of $^{86}Rb^+$ uptake in canine left ventricle. Dog ventricular muscle slices were incubated in 10 ml of K^+-free Krebs-Henseleit solution, containing 2 mM RbCl with a trace of $^{86}Rb^+$ at 30°C. At the indicated time intervals the tissue slices were counted for $^{86}Rb^+$ after a brief washing as described in the methods section. Rb^+ uptake is expressed as the ratio of Rb^+ in tissue to Rb^+ in medium (T/M ratio). Rb^+ uptake was conducted in the absence of 10^{-4} M ouabain in the control (●) or the presence of 10^{-4} M ouabain (□). The difference in Rb^+ uptake assayed in the presence and absence of 10^{-4} M ouabain was considered the active transport of Rb^+. The incubation was also conducted in the presence of 1 mM iodoacetic acid (△) and at 0°C (○) to test energy-dependency and temperature-sensitivity of the Rb^+ uptake system. The vertical bars indicate s.e. and each point represents mean of 6 to 10 determinations.

analysis was performed by use of Student's *t* test and differences are considered significant if probability is lower than 0.05.

Results

Histological studies

Isolation of Purkinje fibres (false-tendon) was quite simple since they are free-running. However, the isolation of SAN and AVN in pure form is impossible as indicated in the methods section. Therefore, as many as 50% of total tissue blocks for each nodal tissue were randomly selected and examined under a light microscope. Usually a few samples out of 10 to 20 stained tissue blocks were contaminated with non-nodal tissue. Both SAN and AVN consisted of a loosely bound connective tissue mass which is distinctly different from the vascular or contractile tissue. For the qualitative detection of such contamination, examination with an electron microscope was not needed. A few contaminated tissue blocks per 10 to 20 random samples indicated that the contamination was not more than 10%.

$^{86}Rb^+$ uptake studies

The characteristics of Rb^+ uptake in the left ventricular tissue of dogs is shown in Figure 1. Total Rb^+ uptake assayed in the absence of ouabain was dependent upon time of incubation as shown by the increase in T/M ratio. Non-specific Rb^+ accumulation was assayed in the presence of 10^{-4} M ouabain. The specific Rb^+ uptake obtained from the difference between the total and the non-specific Rb^+ accumulation was directly linear with incubation time up to 60 min. In order to test whether the Rb^+ uptake system is temperature-dependent, Rb^+ uptake was also carried out at 0°C. Addition of 1 mM iodoacetic acid to the incubation medium decreased Rb^+ uptake to the level of the non-specific accumulation which was assayed in the presence of 10^{-4} M ouabain. The energy-dependent, temperature- and ouabain-sensitive uptake of Rb^+ clearly indicates the uptake system is an active transport process.

A comparison of the specific and the non-specific uptake of Rb^+ in tissue slices of the canine contractile muscles is shown in Figure 2. The non-specific accumulation of Rb^+, assayed in the presence of 0.1 mM ouabain, was similar in all three tissues; this similarity indicates that membrane leakiness to Rb^+ is not greatly different in these tissues. In 21 left ventricular slices obtained from 9 animals, the total and the non-specific uptake of Rb^+ was 4429 ± 325 (s.e.) and 1680 ± 72 pmol per mg tissue (wet weight) after a 30 min incubation. Thus, the specific Rb^+ uptake was 2799 ± 261 pmol/mg tissue of the left ventricle. The specific

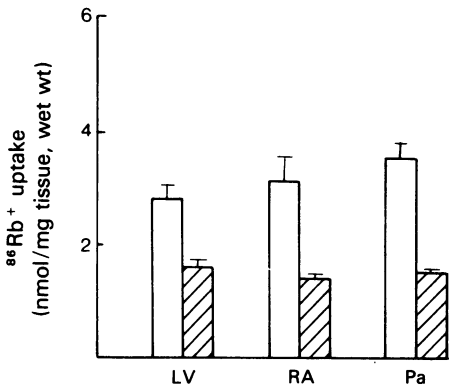

Figure 2 Active transport of Rb^+ in canine left ventricle, right atrium and papillary muscle. The left ventricle (LV), right atrium (RA) and papillary muscle (Pa) from the right ventricle were chopped into relatively homogeneous tissue blocks which were incubated for 30 min to allow uptake of Rb^+ as described in Figure 1. The open columns represent active Rb^+ uptake calculated from the difference between the total and the non-specific accumulation (hatched columns) of Rb^+ assay in the presence of 10^{-4} M ouabain. Each value represents the mean of 15 to 20 determinations and vertical lines indicate s.e. mean.

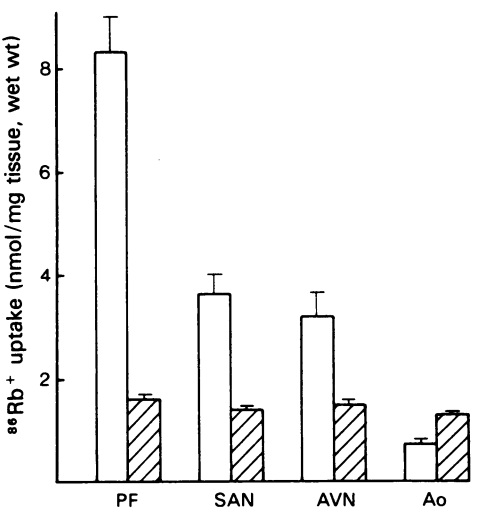

Figure 3 Active transport of Rb^+ in canine Purkinje fibre (PF), sino-atrial node (SAN), atrio-ventricular node (AVN) and aorta (Ao). Rb^+ uptake experiment was conducted for 30 min, as described in Figures 1 and 2. Symbols as in Figure 2. Each value represents the mean of at least 15 individual determinations.

Table 1 Ouabain tissue accumulation after 'therapeutic' and 'toxic' doses in intact open-chest dog hearts

Tissue	*Ouabain accumulation (pmol/g wet wt.)* Therapeutic dose[a]	Toxic dose[b]
Left ventricle	206.9 ± 21.5	504.8 ± 61.6
Papillary muscle	161.9 ± 8.4	399.7 ± 29.6
Right atrium	121.6 ± 17.2	252.8 ± 20.6
Atrio-ventricular node	102.0 ± 19.7	239.6 ± 43.2
Sino-atrial node	87.7 ± 23.7	203.4 ± 17.2
Purkinje fibre	67.4 ± 13.3	155.9 ± 26.2
Aorta	21.5 ± 1.9	71.3 ± 14.2

Each value represents the mean ± s.e. mean of at least 7 dogs.
[a] Loading dose 20 μg/kg, infusion dose 36 ng kg^{-1} min^{-1}. [b] Loading dose 40 μg/kg, infusion dose 72 ng kg^{-1} min^{-1}.

uptake of Rb^+ in the right atrium was not significantly different ($P>0.05$) from that either in the left ventricle or the papillary muscle of the right ventricle. The specific Rb^+ uptake in the papillary muscle appeared significantly ($P<0.05$) greater than the Rb^+ uptake in the left ventricle.

Although the non-specific accumulation of Rb^+ was almost identical in several specialized cardiac tissues (Figure 3), the specific Rb^+ uptake in the Purkinje fibres was almost two to three fold greater than that obtained from other specialized tissues such as the SAN or the AVN. The specific uptake of Rb^+ in the SAN (3693 ± 350 pmol/mg) was not statistically different from that in the AVN (3261 ± 390). The specific uptake of Rb^+ in the ascending aorta was only one tenth of the value obtained from the Purkinje fibre (711 ± 82 vs. 8365 ± 717 pmol/mg, wet weight). The specific uptake of Rb^+ is much lower than the non specific accumulation in this tissue: this low specific uptake indicates that the active Rb^+ transport must be operating very slowly in this vascular tissue.

[^3H]-*ouabain accumulation* in vivo

In order to test the possibility that the differential accumulation of [^{3}H]-ouabain by the functionally different cardiac tissues may account for such differential uptake of Rb^+, [^{3}H]-ouabain tissue content was assayed after an infusion of ouabain as described in the methods section. The highest accumulation of [^{3}H]-ouabain was observed in the left ventricle with both the 'therapeutic' and 'toxic' doses of ouabain (Table 1). In general the contractile muscles such as ventricles and papillary muscle took up more ouabain than the specialized cardiac tissues. Even though the Purkinje fibre had the highest active uptake of Rb^+ (Figure 3), this conducting fibre took up the least amount of ouabain except the ascending aorta. This finding negates the possibility that a greater electrophysiological sensitivity to ouabain in the Purkinje fibre is due to the greater tissue accumulation of this drug in the Purkinje fibre than in the ventricle. When the tissue accumulation of ouabain or the Rb^+ is given as pmol drug per mg protein, instead of mg tissue weight, the overall picture is not really altered (data not shown).

Discussion

Functionally different cardiac tissues have different morphological and pharmacological characteristics. The sino-atrial node (SAN) consists of small, oval shaped, so-called P-cells in a cluster with a few mitochondria and contractile filaments (Cranefield, 1975; Sherf & James, 1979). The slow Ca^{2+} inward current must play a significant role in the pacemaking activity of the SAN, especially in the diseased state (Cranfield, 1975). High extracellular K^+ or tetrodotoxin had little or no effect on the pacemaking property of the node, which is very different from the Purkinje fibre or the ventricular muscle. Large elongated cells in bundles form the Purkinje fibres and have the same maximal diastolic potential as the ventricle (−90 mV). The fact that the Purkinje fibres fire slowly but spontaneously make them quite distinct from the contractile tissue.

The electrophysiological sensitivity to cardiac glycosides such as ouabain in these functionally distinct tissues is well documented (see Introduction). However, the physiological and pharmacological basis of the different sensitivity to ouabain in functionally different cardiac tissues is not known. Furthermore, the distribution of ouabain in the impulse generating nodal tissues or the impulse conducting Purkinje fibre is largely unknown, although the concentration of ouabain in the atria or the ventricles has been documented in relation to the therapeutic and toxic actions of the drugs (Luchi, Park & Waldhausen, 1971; Rhee *et al.*, 1976; Dutta *et al.*, 1968). Therefore, the primary objective of the investigation was to determine the accumulation of ouabain in several specialized canine cardiac tissues. Since the amount of ouabain accumulation in a specific

tissue may be related to the ouabain-sensitivity of the tissue, another important objective of this study was to test whether there is a correlation between the amount of ouabain accumulated and the active uptake of Rb^+ in specialized cardiac tissues. It is not unreasonable to examine the possibility that differential uptake of ouabain by the functionally different cardiac tissues might be related to differential ouabain sensitivity and differential uptake of Rb^+ by the tissues.

The specific loading and infusion doses of ouabain produced plasma concentrations of ouabain of 10^{-8} M with the 'therapeutic' dose and 3×10^{-8} M with the 'toxic' dose as reported previously (Rhee *et al.*, 1976). With constant plasma levels of ouabain for 5 h, tissue accumulation of ouabain in the specialized tissues such as SAN, AVN or Purkinje fibres was significantly lower than the tissue content of ouabain in most of contractile tissues (Table 1). This finding does not support the contention that a high electrophysiological sensitivity of the Purkinje fibre to cardiac steroids is responsible for a greater uptake of the drugs. The present study is in agreement with the finding of Hammerman, Herandez & Goldring (1971) who compared the uptake of digoxin in the bundle of His and the ventricular muscle of the sheep.

Hougen & Smith (1978) studied [^{3}H]-ouabain accumulation in canine left ventricular biopsy sample after an injection of 30 μg/kg of ouabain, which is the middle of our 'therapeutic' and 'toxic' doses of ouabain. They found that the ouabain tissue content was 296 pmol/g tissue; this accumulation is also about midway between the two values obtained from the specific dose regimens used in this study (Table 1). A smaller accumulation of ouabain was reported by Luchi *et al.* (1971) after a brief exposure to ouabain (46 μg/kg); this small accumulation indicates that the *in vivo* tissue accumulation process for ouabain in intact tissue must be very slow in comparison to the *in vitro* binding process of ouabain to purified Na^+, K^+-ATPase (Schwartz, Lindenmayer & Allen, 1975; Rhee & Hokin, 1979).

However, as stated in the methods section the SAN or AVN does not indicate a 'pure' node but includes tissue surrounding the nodes. Although microscopic observation indicates this is quite unlikely, there might be a considerable contamination of the myocardial tissues near the nodes. According to a conservative estimate, over 90% of the nodal preparation consisted of typical nodal tissue devoid of the dense contractile filament-rich myocardium. Since the Purkinje fibre used in this study was free-running false tendon, a contamination of other tissues was not possible. The low uptake of ouabain in the Purkinje fibre may be true rather than due to accidental contamination of other tissues. The possible difference in tissue size and the three dimensional shape of tissue blocks may also contribute to a variation in Purkinje fibres since they are cylindrical strands. Because the tissues were washed at least three times at the end of the incubation, ouabain trapped on the outer surface of the tissue would come off. Moreover, the tissue chopper (Brinkman Instrument, Westbury, N.Y.) produced quite homogeneous tissue blocks by chopping the thin slice of tissue horizontally and vertically.

Because it was sensitive to incubation temperature and was inhibited by ouabain specifically (Figure 1), the $^{86}Rb^+$ uptake system in the present study was active transport process. The uptake system was also dependent upon metabolic energy since iodoacetic acid (1 mM IAA) inhibited the uptake of Rb^+. Nonspecific accumulation of Rb^+ (determined in the presence of 10^{-4} M ouabain) was quite similar in seven different tissues tested in the study (Figures 2 and 3). However, specific uptake of Rb^+ was remarkably different from one tissue to another. The most outstanding fact is that the active uptake of Rb^+ in the Purkinje fibre was the highest among the seven different cardiac tissues tested. This high Rb^+ uptake in Purkinje fibre is at least two to three times greater than any other cardiac tissues (Figure 3) from the same animal and confirms the greater active Rb^+ uptake in the Purkinje fibre than in most of the contractile tissues (Polimeni & Vassalle, 1971; Vassalle *et al.*, 1962).

Since active ion pumping capacity in the Purkinje fibre is greater than any other functionally different tissues, it is understandable that cardiac glycosides inhibit the pump severely in this tissue. The onset of the inhibitory effect of cardiac steroids on the ion pump may occur earlier in the Purkinje fibre than in other tissues. Therefore, the orderly fluxes of ions which are essential for the rhythmic discharge of impulses may be disturbed primarily in the Purkinje fibre. This high capacity of Rb^+ transport in the Purkinje fibre may well explain the early toxicity of cardiac steroids in the fibre (Vassalle *et al.*, 1962). A recent study indicates that the mechanism of digitalis-induced electrical toxicity appears quite different from the mechanism of digitalis toxicity in mechanical contraction (Vassalle & Lin, 1979). Strophanthidin-induced electrical toxicity does not depend exclusively on Ca^{2+} overload. Since a toxic dose of digitalis inhibits Na^+, K^+-ATPase (Lee & Klaus, 1971; Rhee *et al.*, 1976; Schwartz *et al.*, 1975), the increased intracellular concentration of Na^+ can be related easily to the arrhythmogenic mechanism of digitalis. If the role of Na^+ in digitalis-induced toxicity is correct, then it is reasonable to assume that a greater accumulation of Na^+ by the inhibition of the active pump in the Purkinje fibre will produce a greater electrophysiological sensitivity of this tissue to cardiac glycosides. Experimental evidence that supports such a notion is abundant. For instance, at the same concentration of ouabain, the Purkinje fibre became inexcitable earlier than the ventricle from the same heart and

only the Purkinje fibre developed an increased diastolic depolarization after an exposure to the drug (Nowak & Haustein, 1976). Moe & Mendez (1951) reported that intraventricular conduction was depressed much earlier in the Purkinje fibre than in the ventricular muscle.

However, the specialized conducting fibres have a greater water content than the contractile muscles (Vick *et al.*, 1973). This difference was recently confirmed by a new method derived from voltage and cell volume relationship (Houser & Freeman, 1979). As much as 51% of the Purkinje fibre was extracellular space as compared with only 23% of the contractile papillary muscle. Thus, the dry weight of the Purkinje fibres may be far less than the dry weight of the ventricle. Even though this inference is correct, the uptake of ouabain per unit mass of dry weight in the Purkinje fibres will not exceed the uptake of ouabain in the ventricular muscle.

This work was supported in part by grants from NIH, Heart, Lung and Blood Institute (HL-21196) and the North western Ohio Heart Association. The author thanks A.I. Constantinou and M.S. Chang for their competent technical assistance. Reprint requests to H.M.R. in Oklahoma, please.

References

BERNSTEIN, J.C. & ISRAEL, Y. (1970). Active Transport of $^{86}Rb^+$ in human red cells and brain slices. *J. Pharmac. exp. Ther.*, **174**, 323–329.

CLARK, G. (1973). *Staining Procedure Used by the Biological Stain Commission.* 3rd Edition. pp. 33–49. Baltimore: Williams and Wilkins Co.

CRANFIELD, P.F. (1975). *The Conduction of the Cardiac Impulse.* Mount Kisco, New York: Futura Publishing Co.

DUTTA, S., GOSWAMI, S., DATTA, D.K., LINDOWER, J.O. & MARKS, B.H. (1968). The uptake and binding of six radio-labelled cardiac glycosides by guinea pig hearts and by isolated sarcoplasmic reticulum. *J. Pharmac. exp. Ther.*, **164**, 10–21.

HAMMERMAN, H., HERANDEZ, A., & GOLDRING, D. (1971). The uptake of ^3H-digoxin by bundle of his and the myocardium. *J. Lab. clin. Med.*, **78**, 799.

HOUGEN, T.J. & SMITH, T.W. (1978). Inhibitions of myocardial monovalent cation active transport by subtoxic doses of ouabain in the dog. *Circulation Res.*, **42**, 856–863.

HOUSER, S.R. & FREEMAN, A.R. (1979). A simple method of volumetric measurements in isolated cardiac muscle. *Am. J. Physiol.*, **236**, H519–H524.

JAMES, T.N. (1962). Anatomy of the sinus node of the dog. *Anat. Rec.*, **143**, 251–256.

KU, D., AKERA, T., PEW, C.L. & BRODY, T.M. (1974). Cardiac glycosides: Correlations among Na^+, K^+-ATPase, sodium pump and contractility in the guinea pig heart. *Naunyn-Schmiedebergs Arch. Pharmac.*, **258**, 185–200.

LEE, K.S. & KLAUS, W. (1971). The subcellular basis for the mechanism of inotropic action of cardiac glycosides. *Pharmac. Rev.*, **23**, 193–261.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the folin phenol reagent. *J. biol. Chem.*, **193**, 265–275.

LUCHI, R.J., PARK, C.D. & WALDHAUSEN, J.A. (1971). Relationship between myocardial ouabain content and inotropic activity. *Am. J. Physiol.*, **220**, 906–910.

MOE, G.K. & MÉNDEZ, R. (1951). The action of several cardiac glycosides on conduction of velocity and ventricular excitability in the dog heart. *Cirulation* **4**, 729–734.

NOWAK, G. & HAUSTEIN, K. (1976). Different toxic effects of ouabain and 16-epigitoxin on Purkinje fibre and ventricular muscle fibres. *Pharmacol.* **14**, 256–264.

POLIMENI, P.I. (1974). Extracellular space and ionic distribution in rat ventricle. *Am. J. Physiol.*, **227**, 676–683.

POLIMENI, P.I. & VASSALLE, M. (1971). On the mechanisms of ouabain toxicity in Purkinje and ventricular muscle fibres at rest and during activity. *Am. J. Cardiol.*, **27**, 622–629.

RHEE, H.M., DUTTA, S. & MARKS, B.H. (1976). Cardiac Na, K-ATPase activity during positive inotropic and toxic actions of ouabain. *Eur. J. Pharmac.*, **37**, 141–153.

RHEE, H.M. & HOKIN, L.E. (1979). Inhibition of ^3H-ouabain binding to purified Na^+, K^+-ATPase by antibodies against the catalytic subunit. *Biochem. biophys. Acta*, **558**, 108–112.

SCHWARTZ, A., LINDENMAYER, G.E. & ALLEN, J.C. (1975). The sodium-potassium adenosine triphosphatase: pharmacological, physiological and biochemical aspects. *Pharmac. Rev.*, **27**, 1–134.

SHERF, L. & JAMES, T.N. (1979). Fine structure of cells and their histologic organization within internodal pathways of the heart: clinical and electrocardiographic implications. *Am. J. Cardiol.*, **44**, 345–370.

VASSALLE, M., KARIS, J. & HOFFMAN, B.F. (1962). Toxic effects of ouabain on Purkinje fibres and ventricular muscle fibres. *Am. J. Physiol.*, **203**, 433–439.

VASSALLE, M. & LIN, C.I. (1979). Effect of calcium on strophanthidin-induced electrical and mechanical toxicity in cardiac Purkinje fibres. *Am. J. Physiol.*, **236**, H689–H697.

VICK, R.L., CHANG, D.C., NICHOLS, B.L., HAZELWOOD, C.F. & HARVEY, M.C. (1973). Sodium, potassium and water in cardiac tissues. *Ann. N.Y. Acad. Sci.*, **204**, 575–606.

WINEGRAD, S. & SHANES, A.M. (1962). Calcium flux and contractility of guinea pig atria. *J. gen. Physiol.*, **45**, 371–394.

(Received June 27, 1980)