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EFFECTS OF ADRENOCHROME ON RAT HEART SARCOLEMMAL ATPase ACTIVITIES*

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Abstract—Effects of adrenochrome on rat heart sarcolemmal ATPase, calcium binding and adenylate cyclase activities were studied *in vitro*. Adrenochrome (1–100 μg/ml) did not affect calcium binding activity. Significant decreases in adenylate cyclase and Ca^{2+} ATPase activities were seen only at a concentration of 100 μg/ml adrenochrome. Adrenochrome depressed Na^+–K^+ ATPase activity in a dose-dependent manner. The inhibitory effect on Na^+–K^+ ATPase of adrenochrome was also observed in sarcolemmal membranes washed with a buffer after treatment with adrenochrome. The percentage inhibition of the enzyme activity was independent over a wide range of pH (6.6–7.8) and concentrations of NaCl/KCl (40/4–100/10, mM/mM). A study on the combined effects of adrenochrome and either ouabain or $CaCl_2$ showed that, unlike ouabain, calcium produced additive inhibition with adrenochrome. Depression of Na^+–K^+ ATPase activity was also observed in sarcolemma isolated from the heart perfused with adrenochrome. Since adrenochrome has been demonstrated to produce myocardial cell damage and contractile failure in the perfused heart, the present experiments provide a possible explanation for the genesis of adrenochrome-induced cardiotoxicity.

It has been demonstrated that catecholamines in high doses produce myocardial necrosis and disturbance of heart function and metabolism [1–5]. Several theories have been proposed to explain this myocardial lesion; these include increased cardiac work and peripheral vasodilation [1, 6], mobilization of free fatty acids [7], intracellular calcium overload [8], high-energy phosphate store depletion [8, 9] and imbalance of electrolytes in the cell [10, 11]. Recently, it has been suggested that oxidation products of catecholamine such as adrenochrome rather than catecholamines *per se*, produce myocardial cell damage [12–16]. Although plasma and tissue levels of adrenochrome in patients with cardiovascular diseases have not been measured, several pathways for the formation of adrenochrome from epinephrine, including catalysis by cardiac muscle enzymes and mitochrondria, are known [17–24].

Adrenochrome and oxidized isoproterenol in concentrations from 10 to 100 μg/ml have been reported to produce cell damage and contractile failure in the isolated perfused rat heart [12–15]. However, the subcellular mechanisms of the actions of adrenochrome on the heart remain unclear. Adrenochrome and related oxidation products have been shown to affect mitochrondrial oxidative phosphorylation [13, 25], tissue oxygen consumption [26], various aspects of glucose metabolism [27–29], and microsomal calcium uptake [13]. Sarcolemma, by virtue of its ability to control cation movements, is thought to play an important role in regulating heart function and metabolism [30, 31]. This regulatory role of sarcolemma has been attributed to the presence of several membrane-bound enzymes, such as adenylate cyclase, Na^+–K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase, in addition to its calcium binding ability. The present experiments were undertaken therefore, to investigate the influence of adrenochrome on sarcolemmal ATPase, adenylate cyclase and calcium binding activities under *in vitro* conditions. The biochemical activities were also monitored in sarcolemma isolated from hearts perfused with adrenochrome.

Male albino rats weighing 300–400 g were decapitated, and the hearts were quickly removed and placed in a cold buffer solution. The heart sarcolemmal fraction was isolated by the hypotonic shock–LiBr treatment method and purified as described elsewhere [32]. The electron microscopic and marker enzymatic studies [33] revealed minimal contamination by cytoplasmic organelles. Isolation of the myofibrillar fraction was carried out according to the method of Solaro *et al.* [34].

Ca^{2+} ATPase activity of the heart sarcolemmal fraction was measured by incubating approximately 50 μg/ml of membrane protein in a medium containing 50 mM Tris–HCl, 4 mM $CaCl_2$ and 4 mM Tris–ATP, pH 7.4, in the absence or presence of 1 mM EDTA. The results for Ca^{2+} ATPase activity with or without EDTA were not different from each other. Mg^{2+} ATPase activity was measured in a medium containing 50 mM Tris–HCl, 1 mM EDTA, 4 mM $MgCl_2$ and 4 mM Tris–ATP, pH 7.4. Total ATP-hydrolysing activity of the heart sarcolemmal fraction was determined in the medium containing

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50 mM Tris–HCl, 1 mM EDTA, 4 mM $MgCl_2$, 4 mM Tris–ATP, 100 mM NaCl and 10 mM KCl, pH 7.4. The difference between the total ATPase and the Mg^{2+} ATPase activities was taken to be due to Na^+–K^+ ATPase activity. Ouabain-sensitive Na^+–K^+ ATPase activity was estimated as the difference in total ATPase activity in the presence and the absence of 2 mM ouabain. After preincubation of the membrane protein at 37° for 3 min, the reaction was started by the addition of 4 mM Tris–ATP and stopped 10 min later by the addition of 12% trichloroacetic acid. For studying the time–course effect of the drug, adrenochrome and ATP were added simultaneously after the preincubation period, and the reaction was stopped at various time intervals. The reaction mixture was centrifuged at 1000 *g* for 10 min and the supernatant fraction was assayed for inorganic phosphate by the method of Taussky and Shorr [35]. In order to eliminate any interference of the drug during measurement of optical density, the above supernatant fraction was passed through a cotton filter. The difference in values of the control optical density with and without cotton filtration was within 2 per cent. Furthermore, the results with cotton filtration were similar to those obtained by using activated charcoal for the elimination of drug interference in P_i determination [36].

The determination of adenylate cyclase activity in the sarcolemmal fraction was carried out by incubating approximately 0.1 mg of sarcolemmal protein in a medium containing 40 mM Tris–maleate buffer, pH 7.4, 8 mM caffeine, 5 mM KCl, 15 mM $MgCl_2$, 20 mM phosphoenolpyruvate, 130 μg/ml pyruvate kinase and 0.4 mM [^{14}C]ATP (0.25 μCi) at 37° according to a procedure described elsewhere [37]. Calcium binding by heart sarcolemmal fraction was performed by incubating approximately 0.15 mg of membrane protein/ml in a medium containing 50 mM Tris–HCl and 0.1 mM $^{45}CaCl_2$, pH 7.4, at 37° for 5 min and the reaction was terminated by the millipore filtration technique [38]. The sarcolemmal preparation employed here does not exhibit ATP-dependent calcium binding or Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase activities [32], and thus these activities were not studied. Total ATPase activity of the myofibrillar fraction (approximately 1 mg protein/ml) was determined in a medium containing 50 mM KCl, 20 mM imidazole, 2 mM $MgCl_2$, 2 mM Tris–ATP, 10 mM NaN_3 and 0.1 mM $CaCl_2$, pH 7.4. The basal ATPase activity of the myofibrillar fraction was determined in the same medium except that 0.1 mM $CaCl_2$ was replaced by 1.6 mM ethyleneglycolbis (aminoethylether) tetra-acetate (EGTA). The difference between the total and the basal ATPase activities was assumed to be due to Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase activity. All the assay conditions for the determination of enzyme activities employed here were optimal, and the sarcolemmal and myofibrillar fractions were used within 1 hr of their isolation.

To study the effects of adrenochrome on the myocardium, rat hearts were perfused with oxygenated, modified Krebs–Henseleit solution according to a method described earlier [12]. After a 15-min period of equilibration, the hearts were changed to medium with or without the desired concentration of adrenochrome. The contractile force was monitored on a Grass polygraph by means of a force-displacement transducer [12]. The adrenochrome perfused hearts were further perfused with 6 ml of cold control medium to remove adrenochrome from the vascular space. The sarcolemmal fractions from these hearts perfused with or without drug were isolated and the biochemical activities were determined by the methods mentioned above. The protein concentration was estimated by the method of Lowry *et al.* [39]. The drug solution was made immediately before use, and there was no change in pH of the medium due

Table 1. Effects of adrenochrome on rat heart sarcolemmal calcium binding, adenylate cyclase and ATPase activities as well as myofibrillar ATPase activities

	Adrenochrome (μg/ml)					
	Control	1	5	10	50	100
Sarcolemma*						
Calcium binding (nmoles Ca^{2+}/mg/5 min)	21.3 ± 3.0	24.7 ± 4.1	24.9 ± 3.2	22.5 ± 4.9	26.4 ± 3.4	25.4 ± 3.2
Adenylate cyclase (pmoles cAMP/mg/min)	260 ± 58.3	271 ± 89.0	271 ± 75.0	264 ± 59.1	248 ± 77.0	221 ± 66.2†
Ca^{2+} ATPase (μmoles P_i/mg/hr)	44.7 ± 4.0	46.5 ± 5.3	47.1 ± 6.4	48.8 ± 4.3	43.6 ± 3.3	36.2 ± 4.4†
Mg^{2+} ATPase (μmoles P_i/mg/hr)	40.1 ± 2.2	39.3 ± 2.0	38.5 ± 2.1	37.2 ± 2.2†	35.2 ± 2.4†	34.4 ± 1.9†
Na^+–K^+ ATPase (μmoles P_i/mg/hr)	14.8 ± 1.3	15.1 ± 1.3	13.0 ± 1.3	11.6 ± 1.1†	6.7 ± 1.1†	2.9 ± 0.7†
Myofibrils‡						
Mg^{2+} ATPase (nmoles P_i/mg/min)	77 ± 2	78 ± 3	77 ± 3	74 ± 3	63 ± 12	76 ± 9
Ca^{2+}-stimulated ATPase (nmoles P_i/mg/min)	229 ± 15	221 ± 16	222 ± 21	220 ± 24	247 ± 13	250 ± 6

* Each value represents the mean ± S.E. of five to seven experiments.
† Significantly different from the control, $P < 0.05$.
‡ Each value represents the mean ± S.E. of four experiments.

to the addition of the drug solution. The results were analysed statistically by Student's *t*-test.

RESULTS

The effects of various concentrations of adrenochrome (1 to 100 μg/ml or 5.5×10^{-6} to 0.55×10^{-3}M) on sarcolemmal ATPase, calcium binding and adenylate cyclase activities were examined, and the results are shown in Table 1. Adrenochrome did not alter calcium binding activity ($P > 0.05$) significantly, whereas adenylate cyclase and Ca^{2+} ATPase activities were decreased significantly ($P < 0.05$) by 100 μg/ml adrenochrome. A small but significant decrease in Mg^{2+} ATPase activity was observed at 10 μg/ml or higher concentrations of adrenochrome. In contrast, sarcolemmal Na^+–K^+ ATPase activity was depressed markedly in a dose-dependent manner by adrenochrome at concentrations from 10 to 100 μg/ml. The activity fell to 20 per cent of the control value at a concentration of 100 μg/ml adrenochrome. The specificity of adrenochrome action on heart sarcolemmal ATPase is apparent from the data reported in Table 1 which shows that adrenochrome had no effect on the myofibrillar Mg^{2+} ATPase and Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase activities.

In order to examine if the effect of adrenochrome on the sarcolemmal Na^+–K^+ ATPase activity was reversible, we measured the enzyme activity after treating the sarcolemmal membrane with either 10 or 50 μg/ml adrenochrome and after washing the treated membrane twice with a buffer. The Na^+–K^+ ATPase activities of the fresh and untreated control preparations were 14.8 ± 1.3 (N = 7) and 12.7 ± 1.3 (N = 5) μmoles P_i/mg protein/hr, respectively. The activities of the washed preparations after treatment with 10 μg/ml and 50 μg/ml adrenochrome were 9.4 ± 1.1 and 5.1 ± 2.4 μmoles P_i/mg protein/hr respectively. These values were not significantly different from those obtained before washing the treated preparation. Although the enzyme activity of the control preparation was slightly lower than that of the untreated fresh preparation, the percentage inhibition of enzyme activities by treatment with adrenochrome was found to be of the same degree as seen in freshly prepared sarcolemma (Table 1).

Since adrenochrome affected Na^+–K^+ ATPase activity to a greater extent than other sarcolemmal enzyme activities measured in these experiments, further study was undertaken to clarify the characteristics of the inhibition of Na^+–K^+ ATPase activity by adrenochrome. Sarcolemmal Na^+–K^+ ATPase

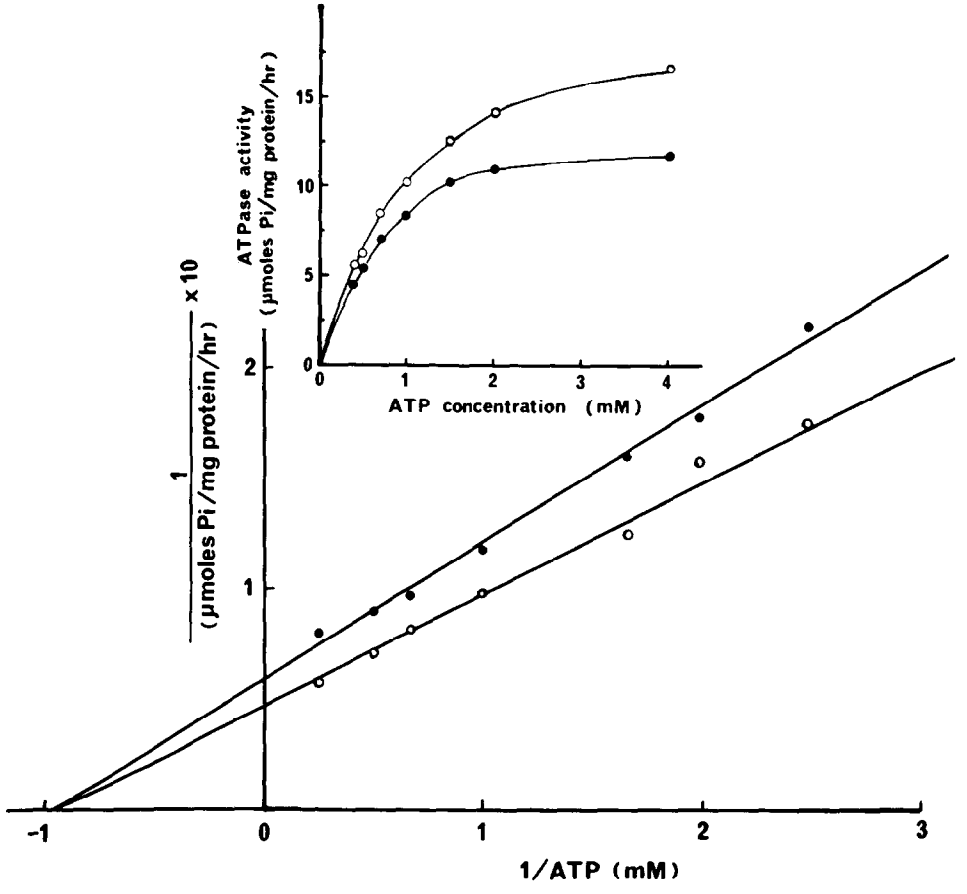

Fig. 1. Rat heart sarcolemmal Na^+–K^+ ATPase activity at different concentrations of ATP in the presence (●) or absence (○) of 10 μg/ml adrenochrome, with Lineweaver–Burk plots. The results are typical of three experiments.

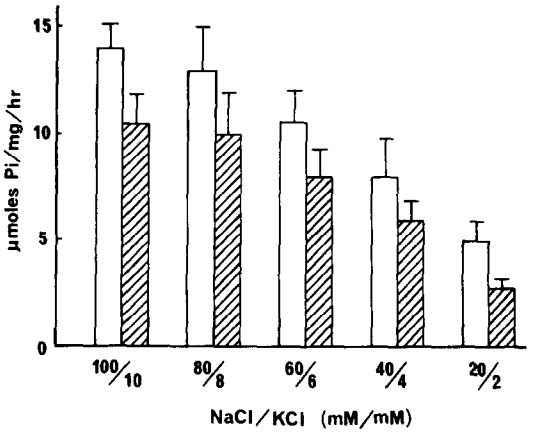

Fig. 2. Rat heart sarcolemmal Na^+–K^+ATPase activity at different concentrations of NaCl/KCl in the presence (▨) or absence (□) of 10 μg/ml adrenochrome. Each value represents a mean ± S.E. of four experiments.

activity was measured at different concentrations of ATP in the absence or presence of 10 μg/ml adrenochrome. Lineweaver–Burk analysis of the data (Fig. 1) showed that the inhibition of Na^+–K^+ ATPase by adrenochrome was associated with a decrease in the V_{max} value without any changes in the K_m value of the enzyme. The enzyme activity was also measured at different pH values and at different concentrations of NaCl/KCl in the incubation medium in the absence or presence of 10 μg/ml adrenochrome. Na^+–K^+ ATPase activity was optimal at pH 7.4 and the inhibition (20–28 per cent) of the enzyme activity by adrenochrome was independent of the pH (6.6–7.8) of the medium. Although Na^+–K^+ ATPase activity was found to be depressed by adrenochrome (Fig. 2) to a greater extent (43 per cent inhibition) at lower concentrations of NaCl/KCl (20 mM/2 mM) than at higher concentrations of NaCl/KCl (approximately 25 per cent inhibition), the significance of this observation is not clear at present. The inhibition was independent of the concentrations of NaCl/KCl from 40/4 to 100/10 (mM/mM). The time-course study concerning the effect of adrenochrome (10 μg/ml) on Na^+–K^+ ATPase activity revealed that the maximal inhibition (about 25 per cent) was apparent within 1 min of incubation.

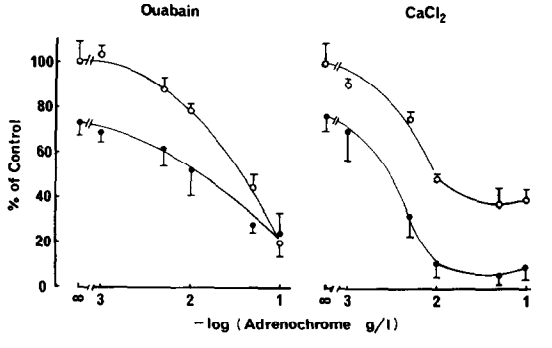

Fig. 3. Interaction of various concentrations of adrenochrome with either 10^{-5} ouabain or 10^{-4}M $CaCl_2$ on rat heart sarcolemmal Na^+–K^+ ATPase activity. Open circles represent Na^+–K^+ ATPase activities in the presence of adrenochrome itself and closed circles, those in the presence of adrenochrome and 10^{-5}M ouabain or 10^{-4}M $CaCl_2$. During the ouabain and $CaCl_2$ inhibition studies, the enzyme activity was measured in the presence and absence, respectively, of 1 mM EDTA. Each value represent a mean ± S.E. of four experiments. The control value of Na^+–K^+ ATPase activity in the ouabain inhibition study was 15.9 ± 1.5 μmoles P_i/mg protein/hr, whereas that in the $CaCl_2$ study was 11.7 ± 2.9 μmoles P_i/mg protein/hr.

The effects of adrenochrome in combination with ouabain and $CaCl_2$, well-known inhibitors of Na^+–K^+ ATPase [40, 41], were studied. Both ouabain and $CaCl_2$ exerted marked depressant effects on heart sarcolemmal Na^+–K^+ ATPase activity; 50 per cent inhibition was seen at 3.5×10^{-5}M ouabain and 2.5×10^{-4}M $CaCl_2$. The effect of different concentrations of adrenochrome was investigated in the absence or presence of either 10^{-5}M ouabain or 10^{-4}M $CaCl_2$, which inhibited the enzyme activity by approximately 20–25 per cent. The results in Fig. 3 indicate that the percentage inhibition of the enzyme activity by 100 μg/ml adrenochrome was virtually identical in the absence or presence of 10^{-5}M ouabain. It was also observed that ouabain-sensitive Na^+–K^+ ATPase (control value 11.1 ± 1.3 μmoles P_i/mg protein/hr; N = 6) was almost completely inhibited by 100 μg/ml adrenochrome (0.8 ± 0.3 μmole P_i/mg protein/hr; N = 6). It should be pointed out that EDTA was omitted when the measurements of the enzyme activity were carried out in the presence of 10^{-4}M $CaCl_2$. It can be seen from Fig. 3 that inhibition patterns for adrenochrome in the absence and presence of EDTA were different from each other. This difference in the responses of the sarcolemmal Na^+–K^+ ATPase to adrenochrome might be due to some contamination of metallic ions in the membrane preparation as well as the incubation medium. Furthermore, the data in Fig. 3 indicate that adrenochrome exerted additive effects when the Na^+–K^+ ATPase activity was measured in the presence of 10^{-4} M $CaCl_2$.

For information concerning the effect of adrenochrome on sarcolemmal Na^+–K^+ ATPase activity in intact cells, isolated rat hearts were perfused with medium containing 50 μg/ml adrenochrome for 30 min. The results in Table 2 indicate that only Na^+–K^+ ATPase activity was depressed significantly ($P < 0.05$) in preparations from hearts perfused with adrenochrome. It was observed that the Na^+–K^+ ATPase activity in preparations from hearts perfused with 50 μg/ml adrenochrome was approximately 25 per cent lower than the control value, whereas this agent *in vitro* produced approximately 55 per cent inhibition of the enzyme activity. This reduced effect might be due to repeated treatments of the membrane preparation with agents such as LiBr and KCl during the isolation and purification procedures. The contractile force of the perfused heart was depressed by approximately 60 per cent of the control value by a 30-min perfusion with 50 μg/ml adrenochrome.

DISCUSSION

In this study we have shown that adrenochrome depressed rat heart sarcolemmal Na^+–K^+ ATPase activity in a concentration range of 10–100 μg/ml.

Table 2. Calcium binding, adenylate cyclase and ATPase activities of rat heart sarcolemma isolated from hearts perfused with or without 50 μg/ml adrenochrome*

	Calcium binding (nmoles Ca^{2+}/mg/5 min)	Adenylate cyclase (pmoles cAMP/mg/min)	Ca^{2+} ATPase	Mg^{2+} ATPase (μmoles P_i/mg/hr)	Na^+–K^+ ATPase
Control	22.5 ± 1.3	267.6 ± 16.2	45.8 ± 3.8	42.3 ± 4.1	14.7 ± 0.6
Adrenochrome	21.9 ± 0.9	287.1 ± 21.8	44.7 ± 1.9	39.5 ± 2.1	10.9 ± 0.4†

* Each value represents the mean ± S.E. of six experiments.
† Significantly different from the control $P < 0.05$.

This inhibitory effect of adrenochrome, particularly at low concentrations, on the Na^+–K^+ ATPase appears to be of a specific nature, since the sarcolemmal Ca^{2+} ATPase, adenylate cyclase and calcium binding activities were not affected by low concentrations of this agent. Furthermore, the percentage inhibition of the sarcolemmal Mg^{2+} ATPase was a little as 15 per cent even at the highest concentration of this agent employed here. The specificity of sarcolemmal Na^+–K^+ ATPase as the site of adrenochrome action is also evident from our results indicating no effect of this agent on ATPase activities of the myofibrillar fraction. Although the inhibitory effect of adrenochrome on sarcolemmal Na^+–K^+ ATPase activity was independent over a wide range of pH (6.6–7.8) and concentrations of NaCl/KCl (40/4–100/10 mM/mM), the kinetic study showed a decreased V_{max} value without any changes in the K_m value of the enzyme. This superficially apparent noncompetitive inhibition may be due to irreversible inhibition or inactivation of the enzyme [42] by adrenochrome.

The experiments reported in this study regarding the interaction of ouabain and calcium with adrenochrome indicate that the inhibitory effect of adrenochrome was additive to that of calcium, whereas Na^+–K^+ ATPase activity was depressed by 100 μg/ml adrenochrome and 10^{-5}M ouabain to an extent similar to that by 100 μg/ml adrenochrome. Furthermore, ouabain-sensitive Na^+–K^+ ATPase was almost completely inhibited by adrenochrome. Although ouabain and calcium are thought to produce inhibitory effects on Na^+–K^+ ATPase activity by acting on different sites [41, 43–46], further experiments are needed to come to a conclusion regarding the exact site of action of adrenochrome. Recently, adrenochrome has been shown to stimulate peroxidation of fatty acids and to modify membrane phospholipids [24]. Whether this membrane effect of adrenochrome is related to the observed changes in sarcolemmal Na^+–K^+ ATPase activity remains an interesting possibility.

Na^+–K^+ ATPase is thought to be involved in the active transport of Na^+ and K^+ across the myocardial cell membrane [14]. An inhibition of this "pump mechanism" can be conceived as resulting in an increase in Na^+ and a decrease in K^+ concentration in the myocardial cell. Such an alteration in the intracellular electrolyte concentration is commonly seen to be associated with heart cell damage and contractile failure and depressed Na^+–K^+ ATPase activity of the sarcolemma has also been reported in different types of failing hearts [31]. The present experiments revealed lower Na^+–K^+ ATPase activity of the sarcolemma obtained from hearts perfused with adrenochrome which showed markedly depressed contractile force. Furthermore, it was interesting to observe that 10–100 μg/ml concentrations of adrenochrome, which are reported to produce contractile failure and cell damage [14, 15], decreased sarcolemmal Na^+–K^+ ATPase activity. Although inhibition of Na^+–K^+ ATPase may explain the adrenochrome-induced cardiotoxicity, it would be premature to arrive at this conclusion at the present time. Our explanation concerning the involvement of Na^+–K^+ ATPase is in contrast to the

common view concerning the mechanism of the positive inotropic effect of cardiac glycosides which decrease Na^+–K^+ ATPase activity [41]. However, cardiac glycosides have been shown to be toxic in concentrations at which these agents inhibit a large amount of the Na^+–K^+ pump activity [41]. In addition other cardiodepressant agents, such as propanolol and several divalent cations, have also been shown to depress heart sarcolemmal Na^+–K^+ ATPase activity [47, 48]. The present study indicating the action of adrenochrome on heart sarcolemma does not exclude the effects of this agent on calcium transport and other activities of heart such as those of the mitochondria and sarcoplasmic reticulum.

REFERENCES

1. G. Rona, C. I. Chappel, T. Balazs and R. Gaudry, *Archs. Path.* **67**, 443 (1959).
2. R. Hill, A. R. Howard and G. S. Gresham, *Br. J. exp. Path.* **41**, 633 (1960).
3. H. Selye, R. Veilleux and S. Grasso, *Proc. Soc. exp. Biol. Med.* **104**, 343 (1960).
4. G. Zbinden, *Am. Heart J.* **60**, 450 (1960).
5. M. Beznak, *Can. J. Biochem. Physiol.* **40**, 25 (1962).
6. C. I. Chappel, G. Rona, T. Balazs and R. Gaudry, *Can. J. Biochem. Physiol.* **37**, 35 (1959).
7. J. K. Kjekshus, in *Pathophysiology and Morphology of Myocardial Cell Alterations* (Eds. A. Fleckenstein and G. Rona), p. 183. University Park Press, Baltimore (1975).
8. A. Fleckenstein, in *Calcium and the Heart* (Eds. P. Harris and L. Opie), p. 135. Academic Press, London (1970).
9. F. Takenaka and M. Higuchi, *J. molec. cell. Cardiol.* **6**, 123 (1974).
10. W. A. Waddell, *J. Physiol., Lond.* **155**, 209 (1961).
11. G. Rona, D. S. Kahn and C. I. Chappel, in *Electrolytes and Cardiovascular Diseases* (Ed. E. Bajusz), p. 181. Karger, Basel (1965).
12. J. C. Yates and N. S. Dhalla, *J. molec. cell. Cardiol.* **7**, 807 (1975).
13. N. S. Dhalla, J. C. Yates, S. L. Lee and A. Singh, *J. molec. cell. Cardiol.* **10**, 31 (1978).
14. G. M. L. Taam, J. C. Yates and N. S. Dhalla, *Can. Fedn biol. Soc.* **19**, 559 (1976).
15. G. M. L. Taam, J. C. Yates and N. S. Dhalla, *J. molec cell. Cardiol.* **9** (suppl.), 55 (1977).
16. E. Severin, S. Sartore and S. Schiaffins, *Experientia* **33**, 1489 (1977).
17. M. D. Hawley, S. V. Tatwawadi, S. Pickarski and R. N. Adams, *J. Am. chem. Soc.* **89**, 447 (1967).
18. R. A. Heacock and W. S. Powell, in *Progress in Medicinal Chemistry* (Eds. G. P. Ellis and G. B. West), Vol. 9, p. 275. North-Holland, Amsterdam (1973).
19. H. P. Misra and I. Fridovich, *J. biol. Chem.* **247**, 3170 (1972).
20. G. Powis, *Biochem. Pharmac.* **28**, 83 (1979).
21. D. E. Green and D. Richter, *Biochem. J.* **31**, 596 (1937).
22. E. Iisalo and A. Pekkarinen, *Acta pharmac. tox.* **15**, 157 (1958).
23. K. Takeshige and S. Minakami, *Biochem. J.* **180**, 129 (1979).
24. S. Gudbjarnason, B. Doell and G. Oskarsdottir, *Acta biol. med. germ.* **35**, 777 (1978).
25. A. R. Krall, G. J. Siegel, D. M. Gozansky and F. L. Wagner, *Biochem. Pharmac.* **13**, 1519 (1964).
26. W. Radsma and H. L. Golterman, *Biochim. biophys. Acta* **4**, 80 (1954).
27. O. Meyerhof and L. O. Randall, *Archs Biochem.* **17**, 171 (1948).
28. I. Pastan, B. Herring, P. Johnson and J. B. Field, *J. biol. Chem.* **237**, 287 (1962).
29. J. Wajzer, *Bull. Soc. Chim. biol.* **29**, 237 (1947).
30. N. S. Dhalla, A. Ziegelhoffer and J. A. C. Harrow, *Can. J. Physiol. Pharmac.* **55**, 1211 (1977).
31. N. S. Dhalla, P. K. Das and G. P. Sharma, *J. molec. cell. Cardiol.* **10**, 363 (1978).
32. N. S. Dhalla, M. B. Anand and J. A. C. Harrow, *J. Biochem., Tokyo* **79**, 1345 (1976).
33. M. B. Anand, M. S. Chauhan and N. S. Dhalla, *J. Biochem., Tokyo* **82**, 1731 (1977).
34. R. J. Solaro, D. C. Pang and N. Briggs, *Biochim. biophys. Acta* **245**, 259 (1971).
35. H. H. Taussky and E. Shorr, *J. biol. Chem.* **202**, 675 (1953).
36. F. Fuchs, E. W. Gertz and F. N. Briggs, *J. gen. Physiol.* **52**, 955 (1968).
37. G. I. Drummond and L. Duncan, *J. biol. Chem.* **245**, 976 (1970).
38. P. V. Sulakhe and N. S. Dhalla, *J. clin. Invest.* **50**, 1019 (1971).
39. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
40. J. C. Skou, *Biochim. biophys. Acta* **23**, 394 (1957).
41. A. Schwartz, G. E. Lindenmayer and J. C. Allen, *Pharmac. Rev.* **27**, 3 (1975).
42. J. Westley, *Enzymic Catalysis*, p. 206. Harper & Row, New York (1969).
43. F. H. Epstein and R. Whittam, *Biochem. J.* **99**, 232 (1966).
44. G. E. Lindenmayer and A. Schwartz, *J. molec. cell. Cardiol.* **7**, 591 (1975).
45. P. V. Sulakhe, D. B. McNamara and N. S. Dhalla, in *The Sarcolemma* (Eds. P. E. Roy and N. S. Dhalla), p. 279. University Park Press, Baltimore (1976).
46. T. Godfraind, A. De Porer and N. Verbeke, *Biochim. biophys. Acta* **481**, 202 (1977).
47. N. S. Dhalla, S. L. Lee, M. B. Anand and M. S. Chauhan, *Biochem. Pharmac.* **26**, 2055 (1977).
48. J. A. C. Harrow, P. K. Das and N. S. Dhalla, *Biochem. Pharmac.* **27**, 2605 (1978).