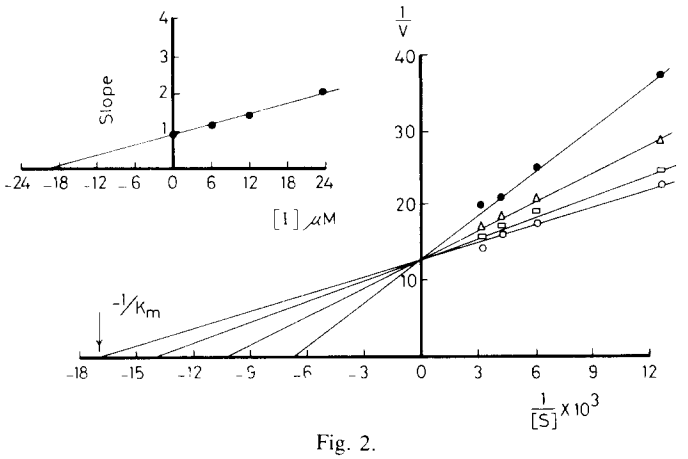

Fig. 2.

brain AChE. The calculated values of K_i and that found from the slope replot are in satisfactory agreement with each other. The Michaelis constant (K_m) is also given in Table 2.

Department of Biochemistry MOHAMED Y. OSMAN
Medical Research Institute
165 Horria Avenue
Alexandria University
Alexandria, Egypt

REFERENCES

1. A. Segal, M. Schroeder, B. Philip and B. L. Van Duuren, *Biochem. Pharmac.* **23**, 937 (1974).
2. T. L. Gresham, J. E. Jansen, F. W. Shaver, M. R. Fredrick, F. T. Fiedorek, R. A. Banker, J. T. Gregory and W. L. Beears, *J. Am. chem. Soc.* **74**, 1323 (1952).
3. H. Raistrick and A. Stossl, *Biochem. J.* **68**, 647 (1958).
4. L. H. Birkenshaw and A. M. L. Dryland, *Biochem. J.* **93**, 478 (1964).
5. Y. S. Mohammed, M. M. Mahfouz and M. Y. Osman, *Biochem. Pharmac.* **26**, 62 (1977).
6. L. W. Harris, J. H. Fleisher, J. Clark and W. J. Cliffs, *Science* **154**, 404 (1966).
7. G. L. Ellman, K. D. Courtney, V. Andres and R. M. Featherstone, *Biochem. Pharmac.* **7**, 88 (1961).
8. Y. S. Mohammed, M. Y. Osman and Y. Gabr, *Arzneimitlet Forsch.* **24**, 121 (1974).
9. M. Dixon, *Biochem. J.* **55**, 170 (1953).
10. T. J. Mantle, K. Wilson and R. F. Long, *Biochem. Pharmac.* **24**, 2031 (1975).

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Cardiovascular and renal properties of potassium-sparing diuretics of the spirolactone group—I: effects of SC-14266/371 (potassium canrenoate) on renal transport Na^+,K^+-ATPase activity

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The maintenance of a high concentration of potassium and low concentrations of sodium inside the cell is the function of the sodium–potassium pump. Much evidence has accumulated suggesting that the transport of sodium and potassium ions across cell membranes is controlled by a specific ATPase system [1]. Evidence that Na^+,K^+-ATPase is the active component of the pump has been strong and overwhelming [2]. It is now widely held that inhibition of the Na^+,K^+-ATPase system by cardiac glycosides is responsible for the positive inotropic effect of these drugs [2].

Spirolactones are of vital importance in the management of patients with congestive heart failure (because of their potassium conservation action), and are used along with cardiac glycosides and other drugs such as the benzothiadiazine-type diuretics. Potassium canrenoate is one such spirolactone, which is water-soluble because of its open lactone ring (Fig. 1). It possesses mineralocorticoid antagonism similar to that of spironolactone in laboratory animals and man [3].

Potassium canrenoate also has a positive inotropic effect in man [4]. Like cardiac glycosides, this drug may be affecting the Na^+,K^+-ATPase activity. We tested this in a series of experiments designed to investigate the action of potassium canrenoate on renal microsomal Na^+,K^+-ATPase activity.

Albino rats of either sex weighing 200–250 g were used in all experiments. Microsomal fractions were prepared by centrifuging 10% kidney homogenates in 0.32 M sucrose/1 mM EDTA, as previously described [5]. ATPase activities were determined by measuring the release of inorganic phosphate from Tris–ATP (4 mmoles/l) in imidazole–HCl buffer (pH 7.4) as reported earlier [6]. All drug solutions were made up in imidazole–HCl buffer. The data presented are the mean of three to seven experiments and the criterion

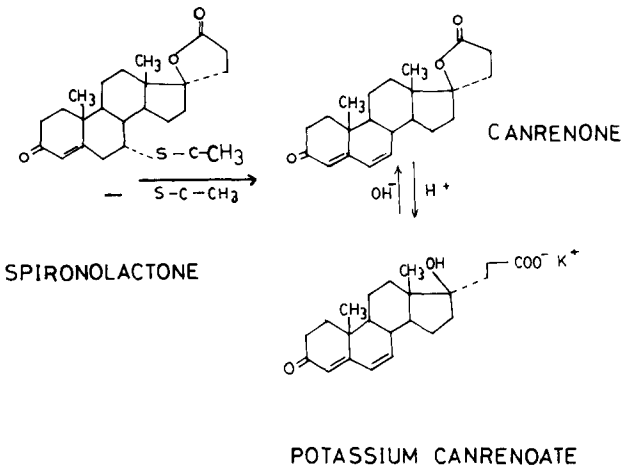

Fig. 1. The formation of canrenone and canrenoate from spironolactone.

for significance was $P < 0.05$ using the Student's *t*-test.

At a concentration of 0.1 mmoles/1, potassium canrenoate significantly inhibited the Na^+,K^+-ATPase activity (Fig. 2), but had no appreciable effect on the Mg^{2+}-ATPase component. By studying a range of concentrations from 0.0125 to 0.1 mmoles/1, it was observed that the concentration used in the experiments shown in Fig. 2 produced maximum inhibitory effects (Fig. 3). It was interesting to note that the renal cortical Na^+,K^+-ATPase activity was also sensitive to the inhibitory action of canrenoate (Fig. 4). This enzyme has previously been shown to be insensitive to the stimulatory action of aldosterone in contrast to the medullary component [6].

A Dixon plot of the results shown in Fig. 3 produced the hyperbolic curve shown in Fig. 5. The upward convex

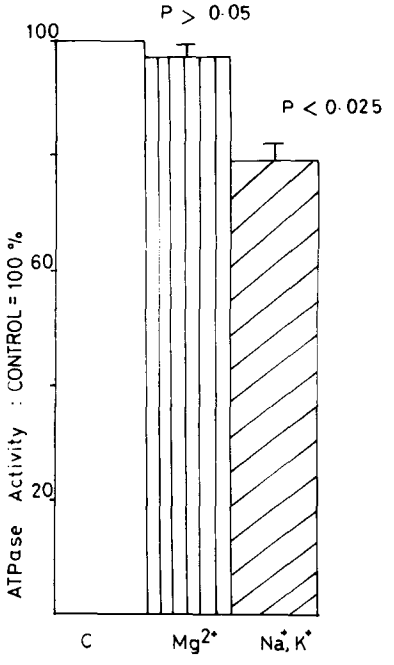

Fig. 2. Effects of SC 14266/371 (potassium canrenoate) at a concentration of 0.1 mmoles/1 on the Na^+,K^+-ATPase and Mg^{2+}-ATPase activities. Activity is expressed as percentage of control. The columns represent means ± S.E.M. of three to seven experiments ($0.01 < P < 0.025$ compared to controls).

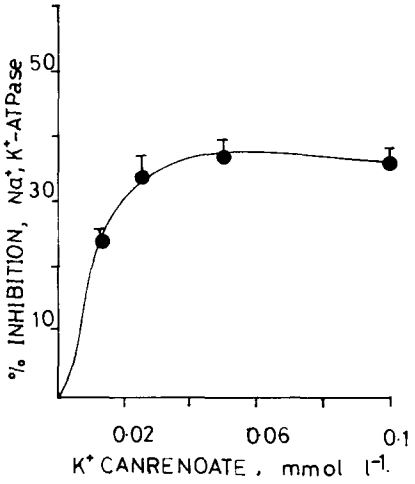

Fig. 3. Dose–response curve for inhibition of renal Na^+,K^+-ATPase activity by potassium canrenoate. Each point is the mean ± S.E.M. of three to seven experiments.

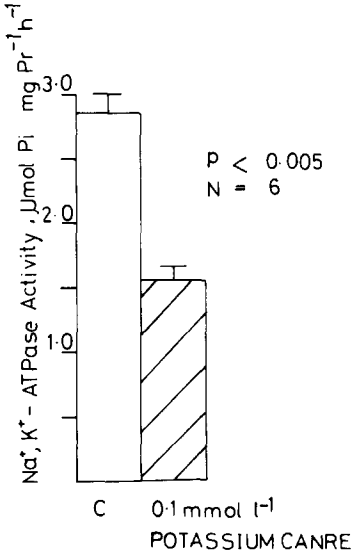

Fig. 4. Action of potassium canrenoate (0.1 mmoles/1) on renal cortical Na^+,K^+-ATPase activity. Each column represents the mean ± S.E.M. of six experiments.

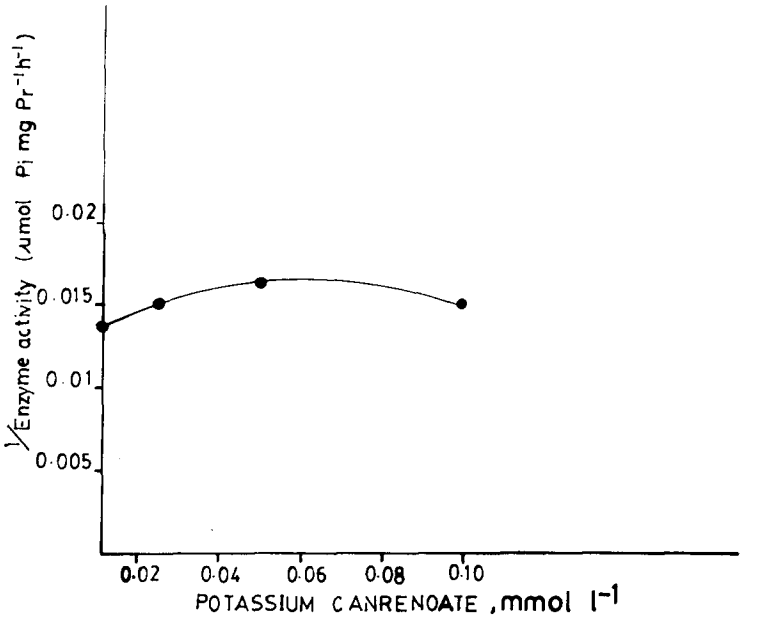

Fig. 5. Dixon plot of inhibition of Na^+,K^+-ATPase activity by potassium canrenoate. Each point is the mean ± S.E.M. of seven experiments.

nature of this curve suggests that we are dealing with a hyperbolic-type of inhibition, according to the nomenclature of Cleland [7].

The studies presented earlier show that potassium canrenoate inhibits the Na^+,K^+-ATPase activity in renal tissue. These results contrast with the observations of Finotti and Palatini [8], who employed brain Na^+,K^+-ATPase and obtained an inhibitory effect of this enzyme with canrenone (the dethioacetylated metabolite of spironolactone), but not with canrenoate.

The nature of the Dixon plot obtained (Fig. 5) suggests that potassium canrenoate may however be only a partial inhibitor of Na^+,K^+-ATPase.

According to Cleland [7], the hyperbolic curve obtained suggests that the ATPase–canrenoate–ATP complex formed can yield inorganic phosphate to a certain extent, though not as effectively as the ordinary ATP–ATPase complex.

Partial rather than complete inhibition of Na^+,K^+-ATPase may explain some of the controversial data in the literature on the pharmacological actions of potassium canrenoate.

Caraboeuf and Deroubaix [9] did not observe an increase in contractility of isolated rat atrial or heart preparations. In contrast, Schroder *et al.* [10] and Waldorff and Buch [4] observed an increased left ventricular contractility in man following intravenous administration of potassium canrenoate. Since the drug appears to partially occupy the Na^+,K^+-ATPase receptor system, the results observed in any system studied would probably depend very much on the concentration used and other factors which might influence the binding of the drug molecules onto the receptor sites.

We have previously reported [11] that the action of aldosterone in increasing renal tubular sodium reabsorption is partly due to stimulation of the Na^+,K^+-ATPase activity. This action is antagonised by spironolactone which itself has no direct effects on the enzyme activity. The present studies suggest that the natriuretic action of canrenoate and the restoration of potassium balance during digitalis therapy may partly be attributed to the action of the drug on the enzyme. The partial antagonism observed with canrenoate in these experiments but not with spironolactone [6] could be due to the fact that canrenoate has an open lactone ring, rather than a closed one as in the case of spironolactone.

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Department of Medical Biochemistry
Faculty of Medicine
University of Nairobi
P.O. Box 30197
Nairobi
Kenya
H. OSORE*

Searle Research and Development
Searle Pharmaceuticals
High Wycombe
U.K.
T. A. HARROW

REFERENCES

1. J. C. Skou, Prog. *biophys. molec. Biol.* **14**, 131 (1964).
2. T. Godfraind, *Biochem. Pharmac.* **24**, 824 (1975).
3. G. M. Kagawa, *Archs int. Pharmacodyn. Thér.* **149**, 8 (1964).
4. S. Waldorff and J. Buch, *Eur. J. Cardiol.* **10**, 143 (1979).
5. H. Osore, *Biochem. Pharmac.* **28**, 2865 (1979).
6. J. G. Gilbert, A. N. Lambie and H. Osore, *Br. J. Pharmac.* **64**, 408 (1978).

* Present address: Chemistry-Bioassay Research Unit, ICIPE Research Centre, P.O. Box 30772, Nairobi, Kenya.

7. W. W. Cleland, *Biochim. biophys. Acta* **67**, 173 (1963).
8. P. Finotti and P. Palatini, *J. Pharmac. exp. Ther.* **217**, 784 (1981).
9. E. Caraboeuf and E. Deroubaix, *J. Pharmac. exp. Ther.* **191**, 128 (1974).
10. R. Schroder, G. Biamino, V. Meyer, B. Ramdohr, W. Sadee and K. P. Schruren, in *Extrarenal Activity of Aldosterone and Its Antagonists* (Eds. W. Brendel, F. J. G. Ebling, T. Friis, G. Greenwood, J. W. Henderson, B. Hokfelt, D. Lauler and R. Witzmann), p. 109. Excerpta Medica, Amsterdam.
11. H. Osore, *E. Afr. med. J.* **58**, 912 (1981).

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Forskolin (a powerful inhibitor of human platelet aggregation)

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Forskolin, a diterpene from the labdane family [1], has been demonstrated to be a powerful stimulant of adenylate cyclase activity in brain and various other tissues [2–5]. We very recently showed that this effect could also be observed in a membrane preparation from human platelets [6]. Forskolin appears to act synergistically with physiological hormonal activators and in an apparently novel manner. From the existing data, it is difficult to conclude definitely whether forskolin acts on the catalytic subunit of adenylate cyclase or needs the presence of a GTP-binding regulatory component [6]. Since the role of cyclic AMP in regulating platelet function is still debated, it was interesting to study the effect of forskolin on platelet aggregation *in vitro* and to correlate the stimulation of adenylate cyclase with the inhibition of aggregation. Because forskolin is a highly hydrophobic compound and may act differently in the two phenomena studied, we used as controls three analogs of forskolin having the same overall structure and hydrophobicity, but devoid of any effect on adenylate cyclase activity.

Materials and methods

EGTA [ethyleneglycolbis(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid], *l*-epinephrine bitartrate creatine phosphokinase, creatine phosphate, dithiothreitol, ionophore A23287, ADP and ATP were obtained from Sigma Chemical Co.; cyclic AMP was from Calbiochem; EDTA (ethylenediaminetetraacetic acid) was from Merck; [α-^{32}P]ATP (21.5 Ci/mmole) was purchased from New England Nuclear. Cyclic [8-^{3}H]AMP (13 Ci/mmole) was obtained from CEA (Saclay, France); bovine fibrinogen was from Piovet. Forskolin was purchased from Calbiochem or as a gift from Hoechst, France. Compounds I and II, derived from manool, were obtained as a gift from Pr. Fetizon (Ecole Polytechnique, Palaiseau, France). Virescenol B was obtained as a gift from Dr Polonsky (Institut de Chimie des Substances Naturelles, CNRS, Gif sur Yvette, France). See Fig. 1 for the chemical structures of the compounds used.

Preparation of washed human platelets. Blood was collected by vein puncture from young male donors who had taken no drugs for 2 weeks, and drawn into a 60-ml syringe containing 2 ml of EDTA (100 mM). Platelet-rich plasma was prepared by differential centrifugation for 10 min at 500 *g* at room temperature [7]. Platelet-rich plasma was then removed and mixed with an equal volume of washing buffer containing 135 mM NaCl, 13 mM sodium citrate, 5 mM glucose and 1 mM EDTA, buffered at pH 7.5. Platelets were then pelleted by centrifugation for 10 min

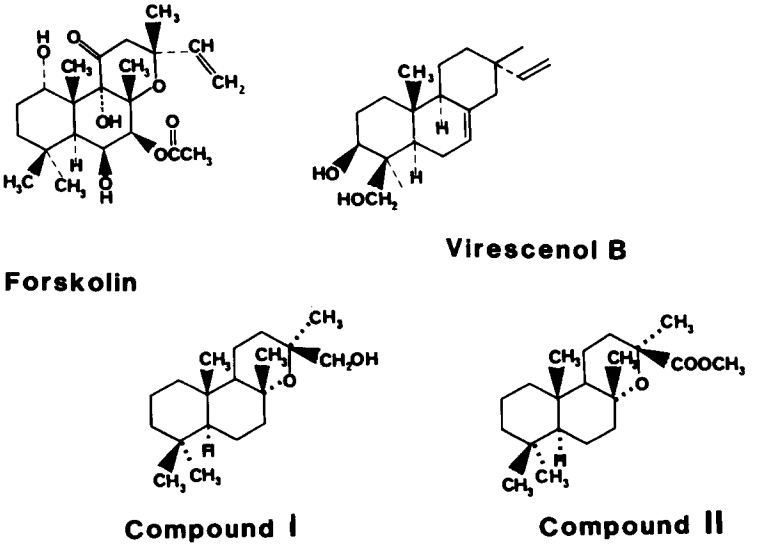

Fig. 1. Chemical structures of forskolin and analogs.