Effect of Phosvitin on Electrocardiographic Changes Produced by Vasopressin in Rats

It is well known that vasopressin constricts coronary arteries[1–14], decreases coronary blood flow in experimental animals[9,14] and consequently myocardial oxygen uptake yielding a coronary insufficiency[9,14,15]. Furthermore it provokes some characteristic electrocardiographic alterations (depressed S-T segment, depressed, flattened or inverted T-wave)[2,5,11,14,16–19] which can be imputed to the myocardial ischaemia[3,18,20].

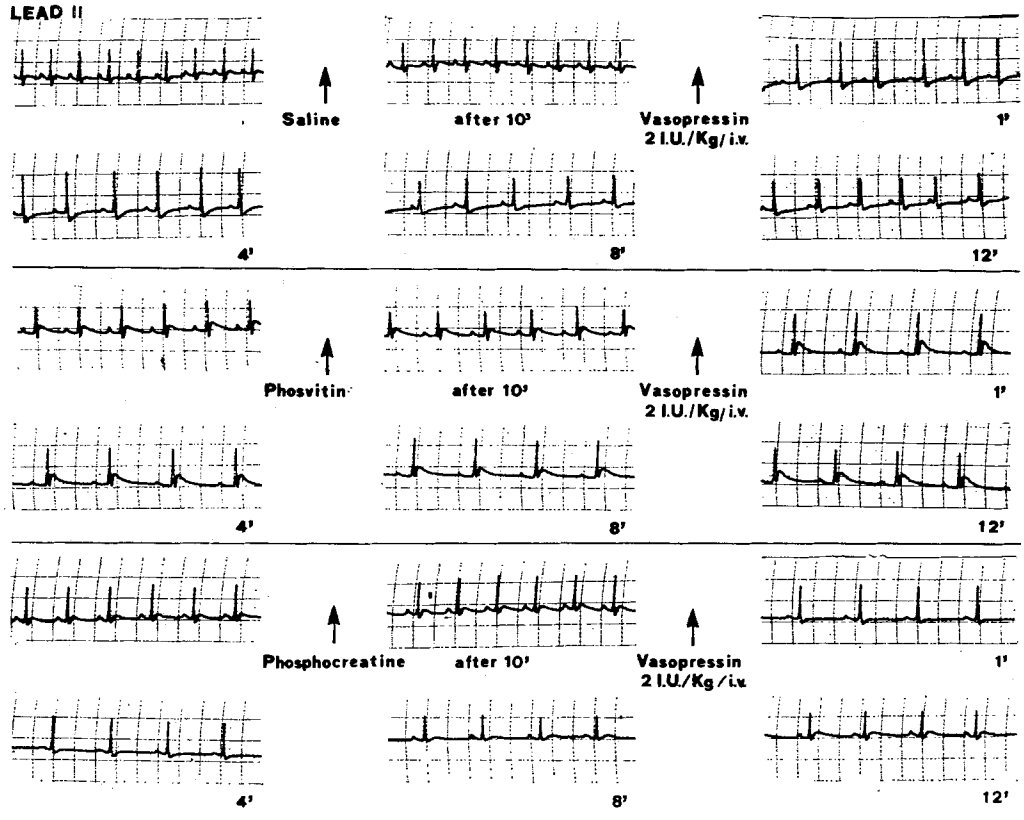
These changes are paralleled by metabolic abnormalities such as a depression of respiratory quotient[14,15] and a shifting of phosphate balance from positive to negative[14] probably because of a breakdown of phosphorilated compounds[14].

On the basis of this knowledge, the problem arose whether compounds with a high content of energy-rich phosphate groups were able to modify the ECG changes produced by vasopressin in rats. In particular phosvitin, an egg-yolk phosphoprotein (M.W. ~50.000; Phosphorous 10%) which can be dephosphorilated by phosphoproteinphosphatase[21,22] and recharged with ATP by a phosphoproteinkinase[23,24] was tested.

Materials and methods. The electrocardiograms on lead II of male Wistar rats (of 250 g) anaesthetized with sodium pentobarbital were recorded.

The following drugs were used: Pitressin (Parke-Davis), Phosvitin extracted from egg-yolk and purified in our

[1] A. Aladov, Trans. Khar'Kov Sci. med. Soc. *1*, 1 (1913).
[2] C. M. Gruber and W. B. Kountz, J. Pharmac. exp. Ther. *40*, 253 (1930).
[3] S. Dietrich, Z. ges. exp. Med. *90*, 689 (1933).
[4] W. Antopol and R. Rössler, Z. ges. exp. Med. *94*, 453 (1934).
[5] K. I. Melville, J. Pharmac. *64*, 86 (1938).
[6] R. Wegria, H. E. Essex, J. F. Herrick and F. C. Mann, Am. Heart J. *20*, 557 (1940).
[7] H. D. Green, R. Wegria and N. H. Boyer, J. Pharmac. exp. Ther. *76*, 378 (1942).
[8] R. Wegria, Pharmac. Rev. *3*, 197 (1951).
[9] K. G. Wakim, C. Denton and E. Essex, Am. Heart J. *47*, 77 (1954).
[10] H. H. Wang, Fedn. Proc. *23*, 565 (1964).
[11] K. I. Melville, H. L. Garvey and R. A. Gillis, Pharmacologist *8*, 174 (1966).
[12] J. Nakano and M. Sakato, Pharmacologist *8*, 474 (1966).
[13] J. Nakano, J. Pharmac. exp. Ther. *157*, 19 (1967).
[14] R. J. Corliss, D. H. McKenna, S. Sialer, G. S. O'Brien and G. G. Rowe, Am. J. med. Sci. *256*, 293 (1968).
[15] V. V. Frol'kis and U. A. Kuz'minskaya, Kardiologia *5*, 41 (1965).
[16] A. Lindner, M. London and G. Werner, Schweiz. med. Wschr. *83*, 360 (1953),
[17] J. W. Black, J. Pharm. Pharmac. *12*, 87 (1960).
[18] P. Fresia, A. Mortari, F. Rognoni and G. Sioli, Archs int. Pharmacodyn. Thér. *152*, 170 (1964).
[19] J. Papp and L. Szekeres, Archs int. Pharmacodyn. Thér. *160*, 147 (1966).
[20] M. Goldenberg and C. J. Rothberger, Z. ges. exp. Med. *76*, 1 (1931).
[21] T. A. Sundararajan and P. S. Sarma, Biochem. J. *53*, 125 (1954).
[22] S. P. Rose and P. J. Heald, Biochem. J. *81*, 339 (1964).
[23] M. Rabinowitz and F. Lipmann, J. biol. Chem. *235*, 1043 (1960).
[24] R. Rodnight and B. E. Lavin, Biochem. J. *93*, 84 (1964).



Effects of Phosvitin and Phosphocreatine on electrocardiographic changes produced by intravenous infusion of vasopressin in rats as compared with control rats. ECG Lead II — 1 mV/cm; chart speed 50 mm/sec.

laboratory, Phosphocreatine disodium salt hydrate (Sigma), DL-Phosphoserine (Fluka), Na_2HPO_4 (Merck), Adenosine-5'-triphosphoric acid disodium salt (Merck). The compounds were used without further purification. All the drugs were dissolved in saline.

The animals received an i.v. infusion of 2 IU/kg of vasopressin at a rate of 1.6 IU/kg/min and phosvitin (750 mg/kg i.p. plus 250 mg/kg i.v. at a rate of 25 mg/kg/min respectively 25 and 10 min before starting the vasopressin infusion). The same schedule was followed with the drugs for comparison, which were administered in stoichiometric amounts relative to the phosphorous content. The ATP was only administered i.v. and in a non-stoichiometric dose (10 mg/kg) because of its side effects (depression of blood-pressure etc.). Control rats, under the same experimental conditions, only received saline in the same volume as used for the other drugs. For each compound at least 9 rats were tested.

Results and discussion. The results are given in the Figure. In all the control rats the ECG alterations as described in the literature were always present. Phosvitin appeared always to exert a protective action of ECG changes produced by vasopressin. In particular T wave was never inverted or flattened; similarly S-T segment alterations were always decreased or absent. On the contrary the comparison drugs phosphoserine, ATP and inorganic phosphates disclosed no activity, with the exception of phosphocreatine which only showed a slight and inconstant activity. The mechanism of protective effect of phosvitin is not known. The physiological role of this protein has been hypothesized as a supplier of energy-rich phosphate[23], an iron carrier[25], or in the oxidative generation of energy-rich phosphate with a reaction in which iron would be involved in some way. The product of this oxidation would be a serine enol-phosphate which can spontaneously liberate its phosphate to charge an ADP by means of an enzymatic system[26]. It has already been demonstrated[27] that phosvitin has no coronarodilator activity and that it does not decrease the blood pressure in the dose which have been employed. These results should be very interesting for any possible future research into the pharmocological aspects of phosvitin.

Riassunto. Viene dimostrata l'attività protettiva della fosvitina sulle alterazioni elettrocardiografiche prodotte dalla somministrazione endovenosa di vasopressina nel ratto. Risultati analoghi non si sono ottenuti impiegando fosfoserina, fosfati inorganici e ATP, fatta forse eccezione per la fosfocreatina per la quale è stata osservata una lieve ma trascurabile attività.

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27 December 1972.

[25] O. GREENGARD, Q. SENTENAC, and N. MENDELSOHN, Biochem. biophys. Acta *90*, 406 (1964).
[26] C. T. GRANT and G. TABORSKY, Biochemistry *5*, 544 (1966).
[27] L. CAPRINO, F. BORRELLI and R. FALCHETTI, unpublished data.
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The Acidic Glycosaminoglycans of the Synovial Fluid in Rheumatoid Arthritis

It is known that the principal acidic glycosaminoglycan (GAG) of synovial fluid in men and other species is hyaluronic acid. The presence of other GAG has been described by other authors[1,2]. Small amounts of chrondroitin-4-sulphate have also been described in some pathological human synovial fluids[3,4], although because of the fractionation methods used it was difficult to quantitate the isomeric chondroitin-sulphates.

This report presents data on the GAG content of the synovial fluid of the knee joint of patients with rheumatoid arthritis and healthy donors using techniques now available for the quantitation of GAG in order to get further insight into the changes in chemical composition of synovial fluid in rheumatoid arthritis.

Material and methods. The synovial fluids were obtained from 18 patients with classical rheumatoid arthritis[5] (males, age 18–39 years) and 12 male donors with apparently normal knews by puncturing the knee joints as described by BALASZ et al.[6]. All patients were not under corticosteroid treatment when samples were taken. As much fluid as possible was obtained from each joint (0.4 to 0.9 ml). Owing to the small amount of fluid obtained from the healthy donors, the respective fluid of 3 joints were pooled. Prior to analysis, samples were centrifuged at $75{,}000 \times g$ to remove the cells and stored at $-20\,^{\circ}C$. The GAG were precipitated by the addition of cetylpyridinium chloride to final concentration of 0.2% and incubated at 37 °C for 1 h.

The crude GAG were purified by dissolving them in $1.25\,M$ magnesium chloride. The resulting GAG were precipitated with 3 volumes of 2% sodium acetate in 95% ethanol during 24 h. Further purification was obtained by redissolving the GAG in 5% potassium acetate and reprecipitating them with 3 volumes of 95% ethanol for 12 h.

The purified GAG thus obtained were dissolved in $0.75\,M$ sodium chloride for further analysis. Total uronic acids were determined on an aliquot of the above by the method of BITTER and MUIR[7] and the original reaction described by DISCHE[8]. Total hexosamine was measured following the method of CESSI and PILLEGO[9] after hydrolysis of aliquot fractions in $4\,N$ HCl at 100 °C for 4 h using glucosamine HCl as standard. Sulphate was determined by an Antonopoulos modification of the benzidine method[10] and nitrogen by the Kjeldahl's method. Galactose was measured with the technique of DISCHE[11]. The purified GAG were then fractionated by chromatography on celulose microlumns by the technique

[1] J. E. SMITH, G. T. CROWLEY JR. and R. B. GILES JR., Arthritis Rheum. *3*, 409 (1960).
[2] J. SANDSON, Science, N.Y. *155*, 839 (1967).
[3] P. SEPPALA, Duodecim *81*, 1049 (1965).
[4] P. SEPPALA, A. LEHTONEN, J. KARKKAINEN and N. NANTÓ, Clinica chim. Acta *16*, 145 (1967).
[5] Committee of the American Rheumatic Association, Arthritis Rheum. *2*, 16 (1959).
[6] E. A. BALASZ, D. WATSON, I. F. DUFF and S. ROSEMAN, Arthritis Rheum. *10*, 357 (1967).
[7] T. BITTER and H. M. MUIR, Analyt. Biochem. *4*, 330 (1962).
[8] Z. DISCHE, in *Methods in biochemical analysis*, (Ed. D. GLICK, Interscience, New York 1955), vol. 2, p. 313.
[9] A. CESI and A. PILLEGO, Biochem. J. *77*, 508 (1960).
[10] C. A. ANTONOPOULOS, Acta chem. scand. *16*, 1521 (1962).
[11] Z. DISCHE, in *Methods in biochemical analysis*, (Ed. D. GLICK, Interscience, New York 1955), vol. 2, p. 327.