CLINICAL RESEARCH

Noradrenaline: a circulating inhibitor of sodium transport

A RIOZZI, A M HEAGERTY, R F BING, H THURSTON, J D SWALES

Abstract

Leucocytes were isolated from venous blood of 11 normotensive volunteers with no family history of hypertension and the sodium efflux rate constants determined both alone and in the presence of increasing physiological concentrations of noradrenaline. There was a significant dose dependent reduction of total sodium efflux rate constant due to a reduction in ouabain sensitive sodium pump activity, glycoside insensitive efflux rate constants being unaffected. The magnitude of this effect was similar to the reduction in leucocyte sodium efflux rate constants observed in hypertensive patients (and their normotensive relatives). The noradrenaline induced depression of sodium pump activity was prevented by propranolol in a further seven experiments, suggesting that the effect was mediated by β adrenoceptors.

Catecholamines possibly functioning as circulating inhibitors of sodium transport may contribute to some of the disturbances in membrane electrolyte handling both in essential hypertension in man and in some experimental models of hypertension.

Introduction

It is generally agreed that ouabain sensitive sodium pump activity is depressed in essential hypertension.¹⁻⁴ The relevance of this is disputed: on the one hand, it has been argued that this abnormality, together with other disturbances of membrane electrolyte handling in hypertension,⁵ is a marker of an inherited global cell membrane abnormality. Another hypothesis links depressed sodium transport to the cellular changes that raise blood pressure⁶ via a circulating inhibitor of sodium pump

activity released in response to expansion of extracellular fluid volume owing to defective renal sodium excretion. The second theory became less attractive when similar depression of sodium transport was found in first degree relatives of patients with hypertension⁷ and when acute alteration of sodium pump activity failed to change blood pressure.⁸

The role of catecholamines in the aetiology of essential hypertension is also uncertain: raised plasma noradrenaline concentrations have been described in some studies but not in others.⁹ A recent study found enhanced vascular reactivity in first degree relatives of hypertensive patients during infusions of noradrenaline¹⁰; in addition, such subjects showed a greater rise in plasma catecholamine concentrations during colour word or cold pressor tests.¹¹ This suggests that catecholamines and depressed active sodium transport might be linked in patients with essential hypertension and their relatives. We have therefore studied the effect of physiological concentrations of noradrenaline on leucocyte membrane sodium transport.

Subjects and methods

Normotensive healthy volunteers were recruited from laboratory staff and students. None had a family history of hypertension, and all were fully informed of the investigation, which was approved by the local ethical committee.

Sodium transport studies were performed on leucocytes obtained from 250 ml blood taken by peripheral venesection from 11 volunteers (10 men) and prepared as described.⁷ In brief, the cells were separated from erythrocytes by differential sedimentation on Plasmagel (Uniscience, Cambridge) and, after washing, residual red cells removed by hypotonic lysis. The cells were then resuspended in their own serum, divided into four aliquots and labelled with sodium-22 (Radiochemicals, Amersham), and allowed to equilibrate for 20 minutes at 37°C. At the end of that time noradrenaline was added to the suspensions to give final concentrations of 0, 2, 4, and 8 nmol/l (0, 0.3, 0.7, and 1.4 ng/ml). The concentrations were selected as being those observed during a cold pressor test.¹¹ After 10 minutes' exposure to noradrenaline the cells were washed and resuspended in serum. Timed samples of each aliquot were then taken in the presence and absence of 0.001M ouabain and the cell pellets counted for ²²Na. Sodium efflux rate constants were calculated by using linear regression analysis to give the slope of the rate of efflux for each aliquot of cells.

In a separate set of experiments leucocytes were obtained from seven different volunteers (five men) and divided into three aliquots. After labelling, the cells were either untreated, or mixed with nor-

University Department of Medicine, Clinical Sciences Building,
Leicester Royal Infirmary, PO Box 65, Leicester LE2 7LX

A RIOZZI, BSC, research assistant

A M HEAGERTY, MB, MRCP, lecturer in medicine

R F BING, MB, FRCP, senior lecturer in medicine

H THURSTON, MD, FRCP, reader in medicine

J D SWALES, MD, FRCP, professor of medicine

Correspondence to: Dr A M Heagerty.

adrenaline in a final concentration of 8 nmol/l, or pretreated with propranolol (10 μ mol/l; 2.96 μ g/ml) and then mixed with noradrenaline (8 nmol/l). Efflux rate constants were calculated as above.

Statistical analyses were performed using Student's paired *t* test and results are expressed as mean and standard error of the mean (SEM).

Results

Mean leucocyte total sodium efflux rate constant was depressed in a dose dependent manner by increasing concentrations of noradrenaline (3.02 (SEM 0.14) *v* 2.70 (0.11)/h at 0 and 8 nmol/l respectively; *p* < 0.05) (fig 1). This depression was due entirely to a significant depression of ouabain sensitive sodium pump activity (2.40 (0.16) *v* 1.89 (0.12)/h; *p* < 0.002), glycoside insensitive efflux rate constants being virtually unchanged (0.62 (0.60) *v* 0.75 (0.12)/h; NS). This suppression of the sodium pump was of the order of 20% and therefore similar to that seen in patients with hypertension and their offspring.^{7, 8}

FIG 1—Effect of increasing concentrations of noradrenaline on mean leucocyte sodium efflux rate constants (*n* = 11). Bars are SEM.

* *p* < 0.05. † *p* < 0.002.

Conversion: SI to traditional units—Noradrenaline: 1 nmol/l $\approx$ 0.17 ng/ml.

Depression of mean leucocyte total sodium efflux rate constant due to reduced sodium pump activity was also found in the second series of experiments with noradrenaline 8 nmol/l (*p* < 0.01; fig 2). This effect of noradrenaline was abolished by pretreatment with propranolol. Thus there was no significant difference in efflux rate constant between untreated leucocytes and those treated with propranolol and noradrenaline (total efflux rate constant 2.8 (0.15) *v* 2.65 (0.22)/h; ouabain sensitive efflux rate constant 2.28 (0.17) *v* 2.10 (0.17)/h). Mean ouabain resistant efflux rate constants were not altered by noradrenaline or noradrenaline and propranolol (fig 2).

Discussion

These results show that noradrenaline in physiological concentrations depresses membrane sodium pump activity in leucocytes. This effect may be abolished by propranolol, suggesting that it is mediated by β adrenoreceptors. The results obtained were higher overall than our previously published values,⁷ reflecting increased sodium efflux obtained when cells

were incubated in serum rather than artificial tissue culture medium. The advantage, however, was that the cells were incubated in a medium similar to that *in vivo*, with endogenous catecholamine and extracellular potassium values held constant. The only change, therefore, was the addition of exogenous noradrenaline.

The degree of inhibition achieved was similar to that seen in leucocytes of hypertensive patients and their normotensive offspring compared with controls. This raises important questions. Firstly, little attention has been paid to the role of

FIG 2—Abolition of noradrenaline induced depression of mean leucocyte sodium efflux rate constants by propranolol (10 μ mol/l; 2.96 μ g/ml) (*n* = 7). Bars are SEM.

* *p* < 0.01.

plasma catecholamines in the reported abnormalities of sodium transport in blood cells. Evidence for raised catecholamine concentrations in hypertensive subjects is controversial, although some degree of increase has been noted in most studies.⁹ Differences between hypertensive subjects and controls may be created or amplified by poor matching and the stress of venepuncture.¹² These factors have not been well controlled in electrolyte flux studies, and simultaneous catecholamine measurements have not been carried out. Alternatively, circulating noradrenaline might still be an important factor in depressed sodium pump activity by its action on cell membranes whose inherited structure is abnormal. Certainly we cannot explain the disturbances in membrane cotransport and countertransport in hypertension⁵ by noradrenaline, since in this study glycoside insensitive pathways were unaltered. Noradrenaline offers an attractive explanation for the depression of ouabain sensitive rubidium uptake found in some experimental models of hypertension.¹³ Such models include that induced by deoxycorticosterone and constricting the renal artery to a single kidney in which catecholamine concentrations are raised.^{14, 15}

By contrast with our observations on leucocytes, it has been shown that hypokalaemia during adrenaline infusion is due to stimulation of skeletal muscle Na⁺, K⁺-adenosine triphosphatase.^{16, 17} Adrenoceptor mediated effects, however, are tissue and species specific. Thus catecholamines stimulate activity of Na⁺, K⁺-adenosine triphosphatase in skeletal muscle of frogs and yet inhibit the sodium pump in cardiac muscle from the same animals.¹⁸

A wide variety of disturbances of cell membrane handling of sodium have been documented in hypertension; moreover, cell membrane structure itself appears to differ, and more recently calcium affinity and extrusion have been found to be abnormal.⁵ It seems most likely that a fundamental disturbance of membrane structure or function, or both, genetically determined, underlies them. Whether this is present in the cells secreting catecholamines or merely in the target organs, thereby altering their response, is uncertain. Nevertheless, the full effects of noradrenaline on cell membrane function in hypertension remain to be elucidated: in addition, the effects of catecholamines on sodium transport will have to be considered more carefully if artefactual findings are to be avoided.

We are grateful to the Trent Regional Health Authority and the Medical Research Council for financing the technical work, and to the Mason Medical Foundation for providing funds for equipment.

References

- 1 Edmondson RPS, Thomas RD, Hilton PJ, Patrick J, Jones NF. Abnormal leucocyte composition and sodium transport in essential hypertension. *Lancet* 1975;ii:1003-5.
- 2 Poston L, Sewell RB, Wilkinson SP, *et al.* Evidence for a circulating sodium transport inhibitor in essential hypertension. *Br Med J* 1981;282:847-9.
- 3 Heagerty AM, Bing RF, Thurston H, Swales JD. Calcium antagonists in hypertension: relation to abnormal sodium transport. *Br Med J* 1983;287:1405-7.

- 4 Gray HH, Poston L, Hilton PJ, Smith SJ, Markandu ND, MacGregor GA. Reversal by verapamil of defect in sodium transport in leucocytes in essential hypertension. *Br Med J* 1984;288:673-5.
- 5 Swales JD. Ion transport in hypertension. *Biosci Rep* 1982;2:967-90.
- 6 de Wardener HE, MacGregor GA. Dahl's hypothesis that a saluretic substance may be responsible for a sustained rise in arterial pressure: its possible role in essential hypertension. *Kidney Int* 1980;18:1-9.
- 7 Heagerty AM, Milner M, Bing RF, Thurston H, Swales JD. Leucocyte membrane sodium transport in normotensive populations: dissociation of abnormalities of sodium efflux from raised blood pressure. *Lancet* 1982;ii:894-6.
- 8 Milner M, Heagerty AM, Bing RF, Thurston H, Swales JD. Changes in leucocyte sodium transport in normotensive relatives of hypertensive subjects: dissociation from blood pressure. *Hypertension* (in press).
- 9 Goldstein DS. Plasma norepinephrine in essential hypertension. A study of studies. *Hypertension* 1981;3:48-52.
- 10 Bianchetti MG, Weidmann P, Beretta-Piccoli C, *et al.* Disturbed noradrenergic blood pressure control in normotensive members of hypertensive families. *Br Heart J* 1984;51:306-11.
- 11 Eliasson K, Hkemdahl P, Kahan T. Circulatory and sympatho-adrenal responses to stress in borderline and established hypertension. *Journal of Hypertension* 1983;1:131-9.
- 12 Reid JL. The autonomic nervous system and hypertension. In: *Advanced medicine* 14. London: Pitman Medical, 1978:70-9.
- 13 Pannani MB, Bugg J, Huot SJ, Haddy FJ. Vascular sodium-potassium pump activity in various models of experimental hypertension. *Clin Sci* 1980;61 (suppl 7):57-60.
- 14 Dargie HJ, Franklin SS, Reid JL. Plasma noradrenaline in experimental renovascular hypertension in the rat. *Clinical Science and Molecular Medicine* 1977;52:477-83.
- 15 de Champlain J, Farley L, Cousineau D, Van Ameringen MR. Circulating catecholamine levels in human and experimental hypertension. *Circ Res* 1976;38:109-14.
- 16 Brown MJ, Brown DC, Murphy MB. Hypokalemia from beta₂-stimulation by circulating epinephrine. *N Engl J Med* 1983;309:1414-20.
- 17 Struthers AD, Reid JL. The role of adrenal medullary catecholamines in potassium homeostasis. *Clin Sci* 1984;66:377-82.
- 18 Buckler KJ, Bhattacharya SS, Flear CTG. Actions of catecholamines and beta-blockers on Na⁺ pump activity in heart and skeletal muscle. *Clin Sci* 1982;63:381.

(Accepted 14 August 1984)

Use of a long acting somatostatin analogue in controlling life threatening ileostomy diarrhoea

N S WILLIAMS, J C COOPER, A T R AXON, R F G J KING, M BARKER

Abstract

A woman presented with persistent ileostomy diarrhoea unresponsive to conventional drug treatment and necessitating parenteral nutrition. Output was four to six litres of watery fluid per 24 hours while she was receiving oral nutrition and two to three litres when she was starved. Treatment with a long acting analogue of somatostatin (50 µg subcutaneously every 12 hours) reduced the ileostomy output to 2.0-2.5 litres/24 hours with an oral diet and the effluent became semiformal. Parenteral fluids could be stopped.

Somatostatin may have a role in the treatment of secretory diarrhoea, but prospective controlled trials are necessary.

Introduction

Somatostatin suppresses many of the gut peptides implicated in the control of secretory and motor activity of the gastrointestinal tract. These actions no doubt contribute to its recently described efficacy in the treatment of patients with bleeding peptic ulcers, the carcinoid syndrome, and enteric fistulas. Its use in the control of diarrhoea has been virtually confined to patients with peptide secretory tumours,¹ although it has been shown to be beneficial in a few patients with profuse diarrhoea due to pseudo-obstruction² and the short bowel syndrome.³ One of the main factors limiting its therapeutic use is its short half life (two to three minutes), and therefore attempts have been made to develop longer acting analogues. We report the successful use of such a drug (SMS 201-995, Sandoz Ltd) to control the output of effluent from a patient with life threatening ileostomy diarrhoea of unknown aetiology.

Case report

A 46 year old woman with Crohn's disease for which she had undergone colectomy and ileorectal anastomosis in November 1980 (35 cm of distal ileum resected) represented in December 1982 with an enterocutaneous fistula due to recurrent disease proximal to the anastomosis. Conservative treatment failed to heal the fistula, and in January 1984 she underwent resection of the fistula, ileorectal anastomosis, and distal ileum (30 cm). A new ileorectal anastomosis was fashioned and defunctioned by creating an end ileostomy. At laparotomy the rest of the small bowel was macroscopically normal.

She recovered from the operation, but during the next four months she had a persistently high ileostomy output, passing four to six litres of watery fluid per 24 hours while receiving oral nutrition and two to

General Infirmary, Leeds LS1 3EX

N S WILLIAMS, MS, FRCS, consultant surgeon, university department of surgery

J C COOPER, FRCS, research fellow, university department of surgery

A T R AXON, MD, FRCP, consultant physician, department of gastroenterology

R F G J KING, PHD, principal biochemist, university department of surgery

M BARKER, BSC, PHD, senior research physicist, department of nuclear medicine

Correspondence to: Mr N S Williams.