British Journal of Nutrition (1988), **60**, 49–55

The effect of Intralipid® infusion on the human leucocyte sodium-pump in vivo

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(*Received 15 October 1987 – Accepted 15 March 1988*)

1. The effect of unsaturated long-chain non-esterified fatty acids (NEFA) on the human leucocyte sodium-pump was studied in vivo.

2. Plasma NEFA level was raised acutely from 0·28 (SD 0·10) to 2·54 (SD 0·48) mmol/l by infusion of 'Intralipid 20%' (trademark) at 90 ml/h with heparin, and the human leucocyte ^{22}Na efflux rate constants were studied in eight normal weight males.

3. After 3 h, there was a significant lowering of the total (from 3·97 (SD 0·92) to 3·10 (SD 0·71)/h; $P < 0·01$) and ouabain-sensitive ^{22}Na efflux rate constants (from 2·89 (SD 0·55) to 2·37 (SD 0·62)/h; $P < 0·02$). Ouabain-insensitive efflux rate constants showed a tendency to fall (from 1·08 (SD 0·51) to 0·73 (SD 0·23)/h). Leucocyte potassium content remained unchanged, but sodium content rose from 31 (SD 12) to 38 (SD 18) mmol/kg dry weight ($P < 0·05$). Total, ouabain-insensitive and ouabain-sensitive efflux rates did not change significantly during the Intralipid-heparin infusion.

4. Plasma insulin levels rose gradually throughout the 3 h infusion period.

5. In conclusion, NEFA, when raised to pathological levels, can inhibit the leucocyte Na-pump in vivo even in the presence of physiological levels of serum albumin, and may increase insulin secretion.

Non-esterified fatty acids (NEFA) have been shown to inhibit Na^+, K^+-ATPase in a variety of animal tissues (Ahmed & Thomas, 1971; Bidard *et al.* 1984; Lamers *et al.* 1984; Tamura *et al.* 1985; Kelly *et al.* 1986). We have previously reported that in normal human beings, there is an inverse correlation between the leucocyte ouabain-sensitive ^{22}Na efflux rate constant (ERC) and the fasting plasma NEFA level (Ng & Hockaday, 1986*a*), and that NEFA, especially when long chain and unsaturated, are potent inhibitors of the leucocyte Na^+, K^+-ATPase in vitro. The concentration of NEFA in fasting plasma is higher than that which can fully inhibit the human leucocyte Na^+, K^+-ATPase. However, albumin binds NEFA (Ashbrook *et al.* 1975) and could lower the free level of NEFA sufficiently to antagonize this inhibitory effect. It is not known whether NEFA play any part in inhibiting cellular Na-pump activity in vivo (Kelly *et al.* 1986) in physiological or pathological conditions.

In human essential hypertension, leucocyte Na-pump activity is reduced (Edmondson *et al.* 1975). Puska *et al.* (1983) showed a reduction in blood pressure by reducing dietary fat intake. Recently, an increased dietary polyunsaturated fat intake in the rat was found to elevate blood pressure and reduce thymocyte ouabain-sensitive ERC (Murray *et al.* 1986), indicating a reduced cellular Na-pump activity. We therefore wished to study the effect on the leucocyte Na-pump of acutely raising plasma NEFA levels in vivo by infusion of 'Intralipid 20%' (trademark of KabiVitrum, Uxbridge, Middlesex), a fat emulsion used for complete intravenous nutrition, and heparin. Any inhibition of the leucocyte Na-pump in vivo would lead to a reduction in the active (or ouabain-sensitive) Na ERC and thereby raise the intracellular Na^+ content.

https://doi.org/10.1079/BJN19880075 Published online by Cambridge University Press

https://doi.org/10.1079/BJN19880075 Published online by Cambridge University Press

SUBJECTS AND METHODS

Materials

Tissue culture fluid TC199 was obtained from Wellcome Diagnostics, Beckenham, Kent, ^{22}Na from Amersham International plc, Amersham, Bucks and the albumin standard, albumin reagent and ouabain from Sigma Diagnostics, Poole, Dorset. 'Intralipid 20%' was from KabiVitrum, Uxbridge, Middlesex, and the sodium heparin from Leo Laboratories.

In vivo studies: the effect of intravenous infusion of Intralipid and heparin on the leucocyte ^{22}Na ERC

Eight normal male subjects (mean body mass index 23·5 (SD 2·6) kg/m^2, age 30·9 (SD 11·9) years) volunteered for the Intralipid infusion study. None had hypertension or any metabolic or endocrine disorder. All subjects gave informed consent. A venous cannula was sited in the antecubital fossa for blood sampling, and a contralateral vein cannulated for infusion of Intralipid. After a period of bed rest for 0·5 h, blood was taken for insulin, glucose, Na and potassium measurements, and 40 ml for leucocyte ^{22}Na efflux studies and intracellular Na and K determinations. The Intralipid infusion (90 ml/h) was started with sodium heparin (200 U immediately and 0·4 U/kg per min thereafter), and blood was taken for insulin, glucose and electrolytes at 30-min intervals for 3 h. At 3 h, measurements of leucocyte ^{22}Na efflux and intracellular electrolytes were repeated.

Leucocyte ^{22}Na ERC and intracellular electrolytes

Leucocytes were isolated by dextran sedimentation, and leucocyte ^{22}Na ERC and intracellular electrolytes were determined as described previously (Ng & Hockaday, 1986*a*). Briefly, the leucocyte pellet was loaded with ^{22}Na (specific activity 1110 kBq/ml) in TC199. After 30 min, the cells were separated by centrifugation and washed once with TC199. Efflux of ^{22}Na was measured in TC199 at 37°, with and without ouabain, 0·1 mmol/l. Portions of the leucocyte suspension were taken every 4 min for 20 min and centrifuged. The radioactivity of the cell pellet and supernatant fraction was measured using a gamma counter, and ERC for total and ouabain-insensitive efflux were determined by plotting $\log_e$ (cellular radioactivity) *v.* time. The ouabain-sensitive ERC is the difference between the total and ouabain-insensitive ERC. The inter-assay coefficient of variation for the ouabain-sensitive ERC was 7%.

Intracellular electrolytes were measured by washing the leucocyte pellet three times in buffered magnesium chloride (95 mmol/l, Tris 5 mmol/l, pH 7·4). The cells were then separated by centrifugation in preweighed tubes, dried to constant weight in an oven, and dissolved in 0·1 mol nitric acid/l after the dry weight was determined (Ng & Hockaday, 1986*a*). Na^+ and K^+ were determined by flame photometry. Inter-assay coefficients of variation for intracellular Na^+ and K^+ were 11 and 9% respectively. Efflux rates were calculated as the product of the ERC and intracellular Na^+.

Insulin was measured by radioimmunoassay (Morgan & Lazarow, 1962) and glucose by a glucose oxidase (*EC* 1.1.3.4) method using a Beckman glucose analyser. Plasma NEFA was determined by an acyl-CoA synthetase (*EC* 6.2.1.3) method (Shimizu *et al.* 1979) and plasma electrolytes by flame photometry. Blood glycerol, acetoacetate and β-hydroxybutyrate were measured in deproteinized samples by enzymic methods (Bergmeyer, 1974). Plasma albumin was measured by a colorimetric method using the albumin reagent and standard albumin solution (50 g/l) from Sigma Diagnostics.

Table 1. *Effect of 'Intralipid 20%' (trademark) infusion on plasma and blood characteristics*

(Mean values and standard deviations)

	Time period after infusion													
	0 min		30 min		60 min		90 min		120 min		150 min		180 min	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
NEFA (mmol/l)	0·28	0·10	1·48	0·51	1·97	0·51	2·28	0·53	2·40	0·52	2·50	0·49	2·54***	0·48
Albumin (g/l)	41·8	2·0	—	—	—	—	—	—	—	—	—	—	40·0	1·2
NEFA: albumin	0·37	0·13	—	—	—	—	—	—	—	—	—	—	3·44**	0·76
Glycerol (mmol/l)	0·06	0·02	0·19	0·06	0·24	0·04	0·26	0·04	0·27	0·03	0·29	0·03	0·30**	0·05
BOH (mmol/l)	0·06	0·03	0·19	0·21	0·34	0·20	0·48	0·28	0·60	0·28	0·73	0·35	0·77**	0·34
AcAc (mmol/l)	0·03	0·01	0·05	0·04	0·07	0·05	0·12	0·07	0·16	0·09	0·19	0·09	0·19**	0·09
Insulin (mU/l)	9·3	3·4	9·1	3·2	10·5	4·2	12·1	4·5	11·1	3·2	11·3	3·8	12·6**	5·2
Glucose (mmol/l)	4·9	0·2	4·7	0·3	4·7	0·3	4·5	0·2	4·7	0·3	4·6	0·3	4·6*	0·3
Na^+ (mmol/l)	140	1	140	2	140	2	140	2	140	2	140	2	140	2
K^+ (mmol/l)	3·9	0·1	3·9	0·1	4·0	0·2	4·0	0·4	3·9	0·4	3·8	0·4	3·7	0·3

NEFA, non-esterified fatty acids; AcAc, acetoacetate; BOH, β-hydroxybutyrate. Mean values at 3 h were significantly different from basal levels (Student's *t* test): * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

Statistics

Statistical analysis of ERC and intracellular electrolytes was by Student's paired *t* test. The changes in plasma NEFA, ketone bodies, insulin, glucose and electrolytes during Intralipid infusion were examined by Student's paired *t* test between levels before and 3 h after starting the infusion. Two-tailed *P* values are given and results are expressed as means and standard deviations.

RESULTS

Intralipid and heparin infusion led to a rise in plasma NEFA level from 0·28 (SD 0·10) to 2·54 (SD 0·48) mmol/l at 3 h after infusion (Table 1). Leucocyte total ERC fell from 3·97 (SD 0·92) to 3·10 (SD 0·71) /h, ($P < 0·01$) while ouabain-insensitive ERC fell from 1·08 (SD 0·51) to 0·73 (SD 0·23)/h (not significant) (Fig. 1). The ouabain-sensitive ERC, which is a measure of Na-pump activity, also fell, from 2·89 (SD 0·55) to 2·37 (SD 0·62)/h, ($P < 0·02$) (Fig. 1). Leucocyte K content remained unchanged (Table 2), but Na content (mmol/kg dry weight) rose from 30·8 (SD 12·1) at fasting to 37·7 (SD 18·1) at 3 h after infusion ($P <$ 0·05, Table 2). The total, ouabain-sensitive and ouabain-resistant efflux rates did not change significantly (Table 2).

The plasma insulin level rose signficantly throughout the course of the infusion (Table 1) and there were the expected rises in plasma NEFA and blood acetoacetate, β-hydroxybutyrate and glycerol levels. This was accompanied by a fall in plasma glucose levels between the first and second hours. Plasma Na and K remained unchanged during the infusion. An infusion of isotonic saline (9 g sodium chloride/l; 90 ml/h) in five normal-weight subjects, did not lead to any substantial changes in leucocyte ^{22}Na ERC (total ERC (/h), from 2·86 (SD 0·27) at fasting to 2·87 (SD 0·69) 3 h after infusion, ouabain-insensitive ERC (/h) from 0·62 (SD 0·07) at fasting to 0·66 (SD 0·30) 3 h after infusion, ouabain-sensitive

https://doi.org/10.1079/BJN19880075 Published online by Cambridge University Press

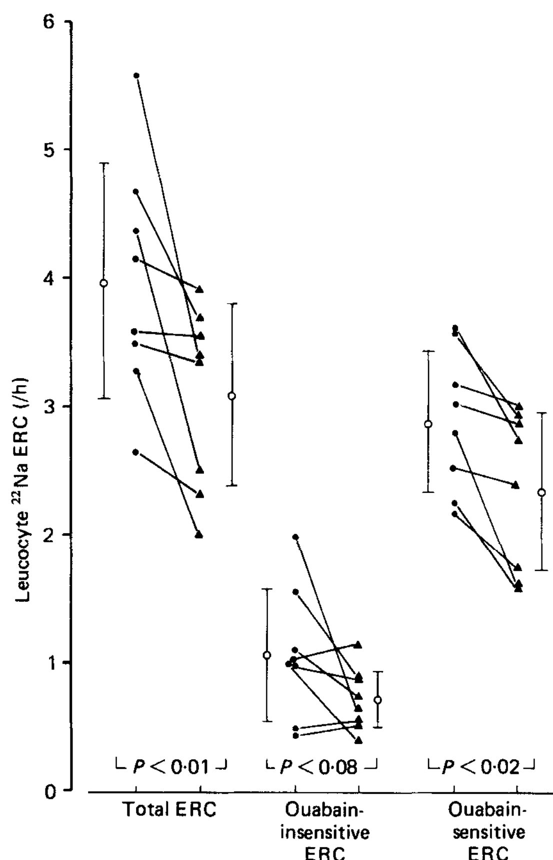

Fig. 1. The effect of 'Intralipid 20%' (trademark) with heparin infusion on leucocyte ^{22}Na efflux rate constants (ERC) in human subjects. (●), Basal; (▲), 3 h after Intralipid–heparin infusion. For details of procedures, see p. 50. Values for individual subjects before and after the infusion are joined by a straight line; (○) mean value, 1 SD represented by vertical bars.

ERC (/h) from 2·24 (SD 0·24) at fasting to 2·21 (SD 0·42) 3 h after infusion), or plasma levels of glucose or insulin (at times 0, 30, 60, 90, 120, 150, 180 min respectively, plasma insulin (mU/l) was 6·3 (SD 3·2), 6·1 (SD 3·1), 6·1 (SD 3·1), 6·9 (SD 3·6), 6·1 (SD 3·0), 5·6 (SD 3·6) and 5·8 (SD 3·1); plasma glucose (mmol/l) was 5·1 (SD 0·4), 5·0 (SD 0·4), 4·9 (SD 0·5), 4·9 (SD 0·5), 4·9 (SD 0·5), 4·9 (SD 0·4), 4·9 (SD 0·5)). Heparin in a concentration of 10000 U/l, acetoacetate concentrations up to 0·2 mmol/l and β-hydroxybutyrate in concentrations up to 2 mmol/l did not inhibit leucocyte Na-pump activity.

DISCUSSION

We have previously reported the inhibitory effect in vitro of long-chain unsaturated NEFA on the leucocyte Na-pump (Ng & Hockaday, 1986*a*), as well as a negative correlation between plasma NEFA concentrations and the leucocyte ^{22}Na ouabain-sensitive ERC from overnight fasting samples. The role of plasma unsaturated NEFA in the control of cellular Na^+ transport in vivo is unknown. However, in the rat, feeding a high polyunsaturated fatty acid diet leads to a higher blood pressure and lower thymocyte ouabain-sensitive ERC compared with normally fed rats (Murray *et al.* 1986).

https://doi.org/10.1079/BJN19880075 Published online by Cambridge University Press

https://doi.org/10.1079/BJN19880075 Published online by Cambridge University Press

Table 2. *Effect of 'Intralipid 20%' (trademark) infusion on leucocyte characteristics*
(Mean values and standard deviations)

Time period after infusion...	0 min		180 min	
	Mean	SD	Mean	SD
Leucocyte:				
Intracellular Na (mmol/kg)	30·8	12·1	37·7*	18·1
Intracellular K (mmol/kg)	347·3	41·5	358·9	44·6
K:Na	12·4	3·3	10·9*	3·5
Leucocyte efflux rates (mmol/kg per h):				
Total efflux	121·9	50·5	115·9	55·9
Ouabain-insensitive efflux	32·6	16·6	26·5	13·2
Ouabain-sensitive efflux	89·2	37·1	89·4	44·7

Mean values were significantly different from basal values: * $P < 0{\cdot}05$.

To test whether this inhibition of the leucocyte Na-pump could be reproduced in vivo, plasma NEFA was raised acutely by Intralipid–heparin infusion. The NEFA composition of the triglycerides found in Intralipid is (%): palmitate 13, stearate 4, oleate 22, linoleate 52, α-linolenate 8, others 1. The present study demonstrated that raising plasma NEFA by an Intralipid–heparin infusion led to a significant lowering of the leucocyte ^{22}Na total and ouabain-sensitive ERC, with a concomitant rise in intracellular Na content. This was probably due to a direct effect of long-chain unsaturated NEFA on Na-pumping by inhibition of the Na^+, K^+-ATPase as described in animal tissues (Ahmed & Thomas, 1971; Lamers *et al.* 1984; Tamura *et al.* 1985) and human leucocytes (Ng & Hockaday, 1986*a*). It is likely that the long-chain unsaturated NEFA led to inhibition of the leucocyte Na-pump as saturated NEFA are less potent inhibitors (Ahmed & Thomas, 1971; Tamura *et al.* 1985; Ng & Hockaday, 1986*a*). Such high levels of plasma NEFA are unlikely to be found normally, except in diabetic ketoacidosis, total parenteral nutrition with Intralipid, and possibly locally in very ischaemic tissues. If inhibition of Na-pumping occurs in the kidney, naturiuresis could result, with the possibility that the natriuresis of fasting could occur via elevation of the plasma NEFA level. Digitalis is known to have a weak diuretic effect, but whether this is a direct effect on the kidney is not known. Further, the hyponatraemia often reported when Intralipid infusion is used for total parenteral nutrition may not be due purely to hypertriglyceridaemia, as it is possible that long-term infusions could lead to substantial cellular Na accumulation.

There was no significant change in the total, ouabain-resistant and ouabain-sensitive efflux rates after Intralipid infusion. At equilibrium, such leucocyte Na^+ efflux rates should equal Na^+ influx rates and, therefore, it may be inferred that little change in leucocyte Na^+ influx resulted from Intralipid infusion. These changes in total and ouabain-sensitive ERC and the inferred changes in Na^+ influx rate confirm the findings in the rats fed on a high polyunsaturated fatty acid diet (Murray *et al.* 1986), although in addition, we found a rise in leucocyte intracellular Na^+ content which Murray *et al.* (1986) postulated but failed to demonstrate significantly.

In essential hypertension, Edmondson *et al.* (1975) found a lowered leucocyte ^{22}Na ouabain-sensitive ERC (reflecting lowered Na^+-pump activity) which led to a raised intracellular Na^+ content. If these changes were also found in blood vessels, a raised blood pressure could result via an increased Na^+-Ca^{2+} exchange (Blaustein, 1977). We found a negative correlation between leucocyte ouabain-sensitive ^{22}Na ERC and fasting plasma NEFA levels (Ng & Hockaday, 1986*a*). The acute effects of increasing plasma unsaturated

NEFA by Intralipid infusion were to raise leucocyte intracellular Na^+ content and lower ouabain-sensitive ERC. We are uncertain of the long-term effects on blood pressure of such changes in cellular ionic transport.

Heagerty *et al.* (1986) showed that increasing dietary linoleic acid led to a rise in ouabain-sensitive Na efflux rates, and, by implication, Na influx may also have increased. Supine blood pressure was reduced in these normotensive volunteers with the linoleic acid supplements. Dietary safflower oil capsules would lead to linoleate-enriched chylomicrons or very-low-density lipoproteins and not to the high plasma linoleate concentrations expected during Intralipid–heparin infusion. The acute effects of raising plasma NEFA on ionic transport could be different from the more chronic effects of increased dietary linoleic acid, when such NEFA are incorporated into membrane lipids.

The rise in plasma insulin with a fall in plasma glucose during Intralipid infusion could be because of a direct stimulatory effect of NEFA on insulin secretion. The rise in plasma insulin is likely to be mediated by a direct effect on the islet β cells, because if it were a result of NEFA leading to peripheral insulin resistance via the glucose–fatty acid cycle, then one would expect a rise in plasma glucose. Similarly, gluconeogenesis from glycerol generated from lipolysis can be discounted. This confirms a previous report (Hicks *et al.* 1977), although others have found the effect inconsistent (Schalch & Kipnis, 1965; Balasse & Ooms, 1973). A possible mechanism for the increased secretion of insulin is an inhibition of Na-pumping in islet cells leading to a rise in intracellular Na. In support of this hypothesis are (a) ouabain leads to insulin secretion from rabbit pancreas slices (Milner & Hales, 1967), an effect abolished by omitting extracellular Na; (b) raising islet cell Na by veratridine provokes insulin secretion (Lowe *et al.* 1976). Another possibility is the stimulatory effect of ketone bodies on insulin secretion, clearly documented in animals (Balasse *et al.* 1967) although difficult to reproduce in man (Balasse & Ooms, 1968). A further explanation could be that the rise in plasma NEFA level led to reduced hepatic clearance of insulin, hence a raised peripheral plasma level of insulin (Stromblad & Bjorntorp, 1986). We have shown that insulin raises this ouabain-sensitive ERC (Ng & Hockaday, 1986*b*). Thus, the small change in plasma insulin during Intralipid–heparin infusion could not have explained the fall in leucocyte ^{22}Na ouabain-sensitive ERC.

In conclusion, we have demonstrated that raising plasma NEFA acutely by infusion of Intralipid and heparin led to inhibition of the leucocyte Na-pump with a rise in intracellular Na^+. Efflux rates remained unaltered. This inhibition may be present in pathological conditions such as diabetic ketoacidosis, locally in ischaemic tissues, and also in total parenteral nutrition with Intralipid, where plasma NEFA levels are raised to high levels. We have also shown a weak but significant stimulation of insulin secretion by this large sustained rise in plasma NEFA level. The effect of polyunsaturated fatty acids on blood pressure and cellular Na^+ transport in human essential hypertension needs further investigation.

This work was financed by the Oxford Diabetes Trust's support of the Sheikh Rashid Diabetes Unit. The authors thank Mrs S. Humphreys, Mr A. Hutchings and Miss E. Kinch for technical assistance. L. L. N was a MRC training fellow.

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https://doi.org/10.1079/BJN19880075 Published online by Cambridge University Press

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Printed in Great Britain

https://doi.org/10.1079/BJN19880075 Published online by Cambridge University Press