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Plasma Insulin Concentrations during Infusion of Potassium and Sodium Chloride Solutions into Conscious Splenectomized Sheep

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Abstract

Haematocrit values, plasma osmolality and the plasma concentrations of sodium, potassium, chloride and insulin were measured in carotid arterial blood before, during and after intravenous infusion of NaCl (0·5 mol l^{-1}) and KCl (0·5 mol l^{-1}) at 2 ml min^{-1} for 105 min into six conscious splenectomized sheep. Hypertonic NaCl infusion was associated with a fall in haematocrit of $1 \cdot 30 \pm 0 \cdot 10\%$ ($P < 0 \cdot 001$) and no consistent change in plasma insulin concentration occurred during this infusion. Hypertonic KCl infusion caused the haematocrit to increase by $1 \cdot 70 \pm 0 \cdot 39\%$ ($P < 0 \cdot 001$) and the plasma insulin concentration to increase by $60 \cdot 0 \pm 16 \cdot 3$ μU ml^{-1} ($P < 0 \cdot 01$). It was concluded that this increase in insulin concentration was caused by elevation of the plasma potassium concentration and was not due to coincident increases in plasma chloride concentration or osmolality. Shrinkage of the extracellular fluid volume during KCl infusion made no major contribution to the increase in insulin concentration which was probably the result of increased release from the pancreas.
[Other keywords: hyperkalaemia, hypernatraemia.]

Introduction

Insulin injections have been found to lower the concentrations of potassium and glucose in the blood of dogs (Briggs *et al.* 1924). Subsequently insulin administration was shown to increase skeletal muscle potassium in rats and man and potassium retention by the livers of normal and potassium-depleted rats (Andres *et al.* 1962; Sitt *et al.* 1966; Mondon *et al.* 1967). Clearly changes in circulatory insulin concentrations in these monogastric animals can alter the rate of uptake of potsssium by cells and thus could be a factor in the control of extracellular potassium concentration if insulin secretion is stimulated by increased extracellular potassium concentration.

Increased potassium concentration in the incubation medium of rabbit pancreatic tissue was found to cause increased insulin release and to enhance insulin release in response to glucose stimulation, but only at potassium concentrations well in excess of normal physiological levels (Hales and Milner 1968; Howell and Taylor 1968). Insulin concentrations in the circulation of anaesthetized and conscious dogs were increased during hyperosmotic infusion of KCl (Santeusanio *et al.* 1971; Hiatt *et al.* 1972*a*; Pettit *et al.* 1975). Plasma glucose concentration fell progressively during increasing hyperkalaemia in the dog (Hiatt *et al.* 1972*b*) which is consistent with the observed increases in insulin concentration, but plasma glucose concentration was not lowered by hyperkalaemia in sheep (Beal 1970). However, ruminants differ from non-ruminants in several aspects of insulin regulation. Plasma insulin levels of sheep are not correlated with plasma glucose concentrations during fasting (Horino *et al.* 1968) and insulin secretion is stimulated by fatty acids having 3–8 carbons (Manns

and Boda 1967; Horino *et al.* 1968). Since plasma glucose concentrations do not necessarily reflect insulin levels in ruminants, no assumptions can be made regarding the effect of hyperkalaemia on plasma insulin concentration in sheep.

The following experiments were designed to establish whether sheep respond to acute hyperkalaemia by increasing the insulin concentration in their circulation and to ascertain whether the concurrent increases in plasma chloride concentration or osmolality caused by KCl infusion are associated with increased plasma insulin concentrations in the whole animals.

Materials and Methods

Experimental Procedures

Six conscious sheep (three Clun Forest ewes weighing 33–56 kg and three Merino crossbred ewes weighing 36–43 kg) were used. Each animal had one carotid artery exteriorized in a loop of skin and all animals had been splenectomized approximately 18 months before being used for these experiments. Splenectomy allows the haematocrit to be used as an indicator of changes in extracellular volume since rapid fluctuations of haematocrit in the sheep are caused by release and sequestration of red blood cells in the spleen. The sheep were maintained on a diet of hay chaff (1000 g daily) and had unrestricted access to water and to a mineral salt supplement.

During the afternoon before the experiment, a vinyl cannula (1·4 mm i.d., 2·0 mm o.d.; Portex Ltd) was inserted under local anaesthesia into one jugular vein using the technique of Seldinger (1953), and a disposable plastic cannula (Braunula, size 0·5; Armour Pharmaceutical Co. Ltd) was introduced into the exteriorized carotid artery. Any uneaten food was removed at this time.

On the morning of the experiment the sheep were transferred to and restrained on canvas stretchers in a normal upright position with their feet just off the floor. An infusion of an isotonic saline solution containing NaCl (145 mmol l^{-1}) and KCl (4 mmol l^{-1}) was commenced via the jugular cannula. After 120 min this isotonic infusion was replaced with a KCl solution (0·5 mol l^{-1}) for the next 105 min followed by the isotonic saline solution for 120 min, an NaCl solution (0·5 mol l^{-1}) for 105 min and the isotonic saline solution for the final 75 min of the experiment. An infusion rate of 2 ml min^{-1} was used for all solutions. Blood samples were taken from the carotid artery during the first period of isotonic saline infusion at 90 and 120 min, during the KCl infusion at 60 and 105 min, during the second period of isotonic saline infusion at 90 and 120 min, during the NaCl infusion at 60 and 105 min, and at the end of the third period of isotonic saline infusion.

Analytical Procedures

Blood samples were taken into plastic syringes containing 1 drop of heparin (5000 i.u./ml) and immediately chilled on ice. Microhaematocrit determinations were made in triplicate within 15 min of sampling on blood spun at 12000 *g* for 10 min in a microhaematocrit centrifuge (Hawksley). The remainder of the blood sample was centrifuged at 2750 *g* for 10 min to obtain plasma which was deep-frozen until needed for analysis. Duplicate estimations of sodium and potassium were made simultaneously by emission flame photometry in an oxygen–propane flame (Autotechnican) with 15 mmol l^{-1} lithium as an internal reference and using mixed sodium and potassium standards in appropriate ranges to correct for mutual interference. The chloride concentration of plasma was measured in duplicate using a Radiometer chloride titrator (model CMT 10). Plasma osmolality was estimated in duplicate by freezing point depression using a Fiske osmometer. Plasma insulin concentrations were measured in duplicate by radioimmunoassay using a double-antibody technique (assay kit provided by the Radiochemical centre, Amersham, England). As human insulin was used to make standards the sheep insulin concentrations obtained by the assay were not absolute values but were human insulin equivalents.

Statistical Procedures

The haematocrit values and the concentrations of electrolytes and insulin in the plasma during the hyperosmotic potassium and sodium infusions were compared to the mean of the values for these variables in the samples obtained before and after each of the hyperosmotic infusions by *t*-test of differences between correlated means (paired *t*-test).

Results

During the intravenous infusion of $0 \cdot 5$ mol l^{-1} NaCl, plasma sodium concentration, chloride concentration and osmolality were all elevated above the values found in plasma during isotonic saline infusion ($P < 0 \cdot 001$, $P < 0 \cdot 001$ and $P < 0 \cdot 01$ respectively), whereas plasma potassium concentration was unaltered by the treatment. Coincident with these alterations in plasma electrolytes the haematocrit fell by $1 \cdot 30 \pm 0 \cdot 10$% (mean ± s.e.). Plasma insulin concentration showed no consistent change during the hyperosmotic NaCl infusion (Fig. 1).

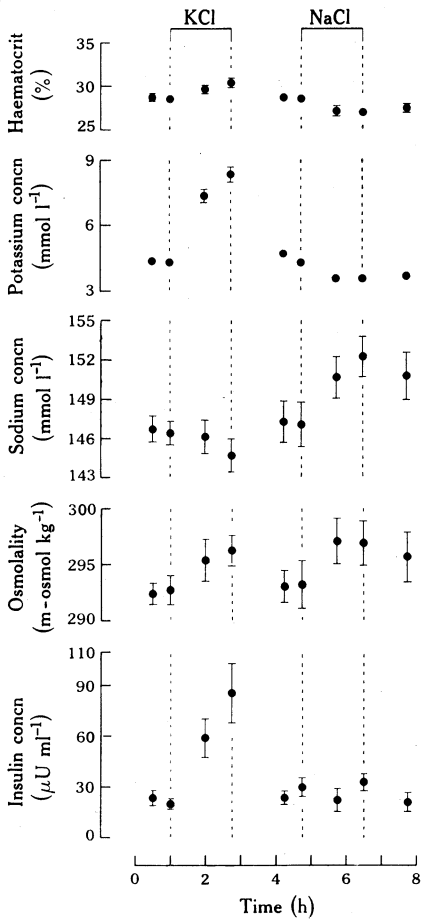

Fig. 1. Plasma potassium and sodium concentrations, plasma osmolality, haematocrit and plasma insulin concentration of carotid arterial blood measured before, during and after intravenous infusions of KCl ($0 \cdot 5$ mol l^{-1}) and NaCl ($0 \cdot 5$ mol l^{-1}) at 2 ml min^{-1} for 105 min into six conscious splenectomized sheep ($n = 6$; means ± s.e.).

When hyperosmotic KCl solution was infused, plasma potassium concentration, chloride concentration and osmolality were increased significantly ($P < 0 \cdot 001$) and plasma sodium concentration fell below the concentration during isotonic saline infusion ($P < 0 \cdot 001$). Plasma chloride concentrations were increased from $106 \cdot 90 \pm 0 \cdot 83$ to $110 \cdot 50 \pm 1 \cdot 05$ mmol l^{-1} and from $107 \cdot 70 \pm 0 \cdot 68$ to $111 \cdot 50 \pm 1 \cdot 12$ mmol l^{-1} by the equimolar KCl and NaCl infusions respectively. The increases in plasma osmolality caused by the two hypertonic infusates were also of similar magnitude. During the potassium infusion haematocrit rose by $1 \cdot 70 \pm 0 \cdot 39$% and plasma insulin concentration increased by $60 \cdot 0 \pm 16 \cdot 3$ μU ml^{-1} (Fig. 1). These increments in haema-

tocrit and plasma insulin were statistically significant ($P < 0{\cdot}005$ and $P < 0{\cdot}01$ respectively). Plasma insulin concentration was positively correlated with plasma potassium concentration throughout all infusions ($r = 0{\cdot}936$; $P < 0{\cdot}001$) but was not correlated with plasma sodium concentration, plasma chloride concentration or plasma osmolality.

Discussion

During the periods of isotonic saline infusion the concentrations of insulin in the arterial plasma of the sheep lay within the normal range which has been previously found for sheep (Bassett 1975). When the hyperosmotic KCl solution was infused the concentration of insulin in arterial plasma was always elevated although the magnitude of this increase was quite variable. Three primary changes in plasma electrolyte composition were caused by the potassium infusion (namely increased potassium and chloride concentrations and increased osmolality). However, the increase in plasma insulin concentration during potassium infusion was not caused by increased plasma osmolality since the infusion of hyperosmotic KCl and NaCl solution increased plasma osmolality to the same extent but only the potassium infusion was associated with increases in insulin concentration. The same argument can be used to eliminate increased plasma chloride concentration as a possible cause of the augmented insulin levels during KCl infusion. Thus the increase in plasma insulin concentration during KCl infusion must be related to the change in plasma potassium concentration.

The increase in plasma insulin during potassium infusion could result from a reduction in extracellular volume and/or a reduction in the clearance of insulin from the plasma and/or increased release of insulin from the pancreas. In these splenectomized sheep the haematocrit can be used as an indicator of changes in extracellular volume. During the potassium infusion the maximum increase in haematocrit ranged from 0·2 to 2·5% which indicates a small fall in extracellular volume owing to redistribution of fluid between the extracellular and intracellular compartments and to the diuretic action of hyperkalaemia in the sheep (Beal *et al.* 1973). At the same time the plasma insulin concentration was augmented 1·9–5·4 times. Thus decreases in extracellular volume made a negligible contribution to the increase in plasma insulin concentration.

No attempt was made to ascertain the relative magnitude of the effects of hyperkalaemia on insulin clearance rates and pancreatic release of insulin in these experiments. However, previous studies in sheep have shown that acute hyperkalaemia, of the level produced in these experiments, increases cardiac output and blood flow to many tissues and causes diuresis (Beal *et al.* 1975; Beal 1976*a*, 1976*b*). All of these effects would favour increased clearance of insulin from the circulation and a reduction in plasma concentration rather than the converse. Thus increased release of insulin from the pancreas probably accounts for most of the increase in plasma insulin levels during acute hyperkalaemia. This interpretation would be consistent with previous observations on isolated rabbit pancreas and perfused rat and dog pancreas (Grodsky and Bennet 1966; Hales and Milner 1968; Howell and Taylor 1968; Kuzuya *et al.* 1974).

This investigation shows that like the dog, the sheep responds to acute hyperkalaemia with an increase in plasma insulin concentration. This may be an important

factor in controlling the rate of increase in the potassium concentration of the extracellular fluid of sheep having high potassium intakes.

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References

Andres, R., Baltzan, M. A., Cader, G., and Zierler, K. L. (1962). Effect of insulin on carbohydrate metabolism and on potassium in the forearm of man. *J. Clin. Invest.* **41**, 108–15.

Bassett, J. M. (1975). Dietary and gastro-intestinal control of hormones regulating metabolism in ruminants. In 'Digestion and Metabolism in the Ruminant'. (Eds I. W. McDonald and A. C. I. Warner.) p. 384. (University of New England: Armidale.)

Beal, A. M. (1970). Potassium excretion in Merino sheep. Ph.D. Thesis, University of Queensland.

Beal, A. M. (1976*a*). Changes in arterial blood pressure, heart rate and haematocrit during acute hyperkalaemia in conscious sheep. *Q. J. Exp. Physiol.* **61**, 297–308.

Beal, A. M. (1967*b*). The effect of intravenous infusion of hyperosmotic sodium and potassium chloride solutions on cephalic blood flow in conscious sheep. *Q. J. Exp. Physiol.* **61**, 309–19.

Beal, A. M., Budtz-Olsen, O. E., Clark, R. C., Cross, R. B., and French, T. J. (1973). Renal and salivary responses to infusion of potassium chloride, bicarbonate and phosphate in Merino sheep. *Q. J. Exp. Physiol.* **58**, 251–65.

Beal, A. M., Budtz-Olsen, O. E., Clark, R. C., Cross, R. B., and French, T. J. (1975). Changes in renal haemodynamics and electrolyte excretion during acute hyperkalaemia in conscious adrenalectomized sheep. *Q. J. Exp. Physiol.* **60**, 207–21.

Briggs, A. P., Koechig, I., Doisy, E. A., and Weber, C. J. (1924). Some changes in the composition of blood due to the injection of insulin. *Biochem. J.* **58**, 721–30.

Grodsky, G. M., and Bennett, L. L. (1966). Cation requirements for insulin secretion in the isolated perfused pancreas. *Diabetes* **15**, 910–13.

Hales, C. N., and Milner, R. D. G. (1968). The role of sodium and potassium on insulin secretion from rabbit pancreas. *J. Physiol. (London)* **194**, 725–43.

Hiatt, N., Davidson, M. B., and Bonorris, G. (1972*a*). The effect of potassium chloride infusion on insulin secretion *in vivo*. *Horm. Metab. Res.* **4**, 64–8.

Hiatt, N., Miller, A., and Lipkins, J. (1972*b*). The effect of potassium chloride infusion on insulin secretion *in vivo*. *Horm. Metab. Res.* **4**, 64–8. Cited by Hiatt *et al.* (1972*a*).

Horino, M., Machlin, L. J., Hertelendy, F., and Kipnis, D. M. (1968). Effect of short-chain fatty acids on plasma insulin in ruminant and non-ruminant species. *Endocrinology* **83**, 118–28.

Howell, S. L., and Taylor, K. W. (1968). Potassium ions and the secretion of insulin by islets of Langerhans incubated *in vitro*. *Biochem. J.* **108**, 17–24.

Kuzuya, T., Kajinuma, H., and Ide, T. (1974). Effect of intrapancreatic injection of potassium and calcium on insulin and glucagon secretion in dogs. *Diabetes* **23**, 55–60.

Manns, J. G., and Boda, J. M. (1967). Insulin release by acetate, proprionate, butyrate, and glucose in lambs and adult sheep. *Am. J. Physiol.* **212**, 747–55.

Mondon, C. E., Burton, S. K., Grodsky, G. M., and Ishida, T. (1967). Glucose tolerance and insulin response of potassium-deficient rat and isolated liver. *Diabetes* **16**, 510.

Pettit, G. W., Vick, R. L., and Swander, A. M. (1975). Plasma K^+ and insulin: changes during KCl infusion in normal and nephrectomized dogs. *Am. J. Physiol.* **228**, 107–9.

Santeusanio, F., Knochel, J. P., Schlein, E. M., Faloona, G. R., and Unger, R. H. (1971). Effect of acute hyperkalaemia on plasma insulin and glucagon. *J. Lab. Clin. Med.* **78**, 1001–2.

Seldinger, S. I. (1953). Catheter replacement of the needle in percutaneous arteriography. *Acta Radiol.* **39**, 368–76.

Sitt, R., Losert, W., Schultz, G., Kaess, H., and Senft, G. (1966). Der Einfluss von Insulin auf den Kalium-transport in der Skeletmuskulatur. *Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol.* **255**, 398–406.

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