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Nitric oxide-related pancreatic endocrine responses to hyperglycaemia in the conscious calf

A. V. Edwards, M. A. Ghatei[a] and S. R. Bloom[a]

Physiological Laboratory, University of Cambridge, Downing Street, Cambridge CB2 3EG (UK) and [a]Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, Ducane Road, London W12 ONN (UK)
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Abstract. Mean plasma insulin concentration was reduced and mean plasma glucose concentration increased following the administration of N-nitro-L-arginine methyl ester (L-NAME; 100 μmol kg^{-1} i.a.) in conscious calves given continuous infusions of exogenous glucose (30–60 μmol min^{-1} kg^{-1} i.v.). It is concluded that the rise in plasma insulin concentration which occurs in these animals in response to glucose is mediated, at least in part, by a nitric oxide-related factor (NOx).
Key words. Nitric oxide; insulin; endocrine pancreas; L-NAME.

NOx has been implicated in the control of insulin release from the islets of Langerhans in vitro both in respect of arginine and glucose-evoked secretion[1] although the question whether it is released within the β-cells themselves and/or from parasympathetic nerve terminals or endothelial cells is controversial[2], as is the question whether NOx is capable of both stimulating and inhibiting insulin release[1,2]. It has been suggested that NOx inhibits insulin release from islet cells following exposure to interleukin-1β[3] and the comparative resistance of human islets to interleukin has been attributed to a relative lack of induction of nitric oxide (NO) synthesis in those cells[4]. In the conscious calf, insulin release in response to exogeneous glucose is reported to be mediated largely via the parasympathetic innervation[5]. Since nitric oxide synthase (NOS) is present in nerve terminals in pancreatic islets, at least in the rat[2], the present study was undertaken to investigate the effect of blocking NO synthesis on the pancreatic endocrine response to hyperglycaemia under strictly physiological conditions in conscious unrestrained calves. The results show that blocking the synthesis of NOx significantly and specifically reduces plasma insulin and raises plasma glucose concentration in animals given exogenous glucose but not otherwise. They would be entirely consistent with a role for NOx as an insulinotropic agonist in vivo.

Materials and methods

The experiments were carried out in conscious pedigree Jersey calves 14–36 days after birth (26.0–34.8 kg b. wt) in which intra-arterial catheters had been emplaced previously under general halothane anaesthesia so that the tips lay in the lower thoracic aorta; braunula catheters were inserted into a jugular vein under local anaesthetic when required for i.v. infusion. In animals given glucose, injection of Dextrose Monohydrate B.P. (50% w/v, HMC [Manufacturing Chemists] Ltd, Dundee) was diluted in an appropriate volume of distilled water for administration at 30–60 μmol min^{-1} kg^{-1} in a volume of 1.0 ml min^{-1} kg^{-1} i.v. NOS was blocked by the administration of N^{w}-nitro-L-arginine methyl ester HCl (Sigma, L-NAME) at a dose of 100 μmol kg^{-1} by an intra-aortic injection above the origin of the arterial supply to the pancreas. The cardiovascular consequences were thereafter minimised by a continuous i.v. infusion of sodium nitroprusside (Sigma) dissolved in physiological saline at a dose of 4–8 nmol min^{-1} kg^{-1}. Aortic blood pressure and heart rate were monitored continuously by means of a Devices M19 recorder.

Samples of arterial blood were collected at intervals into heparinized tubes containing aprotinin (Trasylol; Bayer; 1000 KIU ml^{-1} blood) for glucose and peptide hormone estimations. Each was then centrifuged at 4 °C and the plasma sequestered at −20 °C . Glucose was measured by means of a Mark 2 Beckman Glucose Analyzer. Pancreatic glucagon, insulin and pancreatic polypeptide were measured by radioimmunoassays as described previously[6]. Results are expressed as mean values ± SE of mean. They were analyzed statistically by Student's *t* test or the paired *t* test, as appropriate.

Results and discussion

Administration of L-NAME was followed by a continuous i.v. infusion of sodium nitroprusside at a dose (4–8 nmol min^{-1} kg^{-1}) adjusted to maintain a constant arterial blood pressure and heart rate, as originally described by Bower and Law[7] (fig. 1). No animal displayed any behavioural response to this procedure at any time. L-NAME had no significant effect on the plasma concentration of any pancreatic hormone or on plasma glucose concentration in normal control animals. However, in animals given continuous i.v. infu-

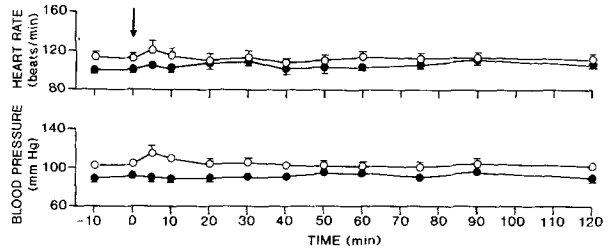

Figure 1. Changes in mean aortic blood pressure and heart rate in response to L-NAME (100 μmol kg^{-1} i.a.; ↓) followed by sodium nitroprusside (4–8 nmol min^{-1} kg^{-1} i.v.) in 6 normal control calves (●) and in 8 calves given exogenous glucose (30–60 μmol min^{-1} kg^{-1} i.v.; ○).

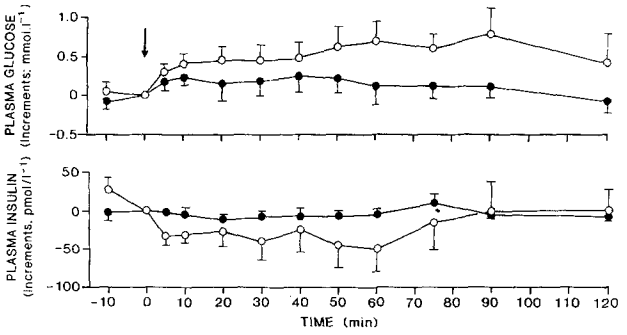

Figure 2. Changes in mean arterial plasma glucose and insulin concentration in response to L-NAME (100 μmol kg^{-1} i.a.; ↓) followed by sodium nitroprusside (4–8 nmol min^{-1} kg^{-1} i.v.) in 6 normal control calves (●) and in 8 calves given exogenous glucose (30–60 μmol min^{-1} kg^{-1} i.v.; ○).

sions of exogenous glucose (30–60 μmol min^{-1} kg^{-1}) which raised the basal plasma glucose concentration from 4.8 ± 0.2 mmol l^{-1} to 7.3 ± 0.2 mmol l^{-1}, administration of L-NAME was followed by a statistically significant fall in mean plasma insulin concentration, which was associated with a significant rise in mean plasma glucose concentration (fig. 2). Thus, the average mean decremental plasma insulin concentration during the 60 min period after L-NAME was -36 ± 4 pmol l^{-1} compared with a mean average value of $+2 \pm 7$ pmol l^{-1} before and after that 60 min period ($p < 0.001$); the mean average absolute values (194 ± 3 pmol l^{-1} during the 60 min after L-NAME and 231 ± 7 pmol l^{-1} before and after it) were also significantly different ($p < 0.001$). There was also a significant rise in mean plasma glucose concentration; each of the values during the same 60 min period was significantly higher than the initial value (7.3 ± 0.2 mmol l^{-1}; $p < 0.025$). Since the fall in plasma insulin concentration could be expected to be self limiting under these conditions, as the rise in plasma glucose concentration would increase the intensity of the hyperglycaemic stimulus to release of the hormone, the ratio of insulin to glucose in the plasma was estimated and found to provide an even more sensitive index of the response to L-NAME, but there was no significant fall in the absence of exogenous glucose (fig. 3). L-NAME

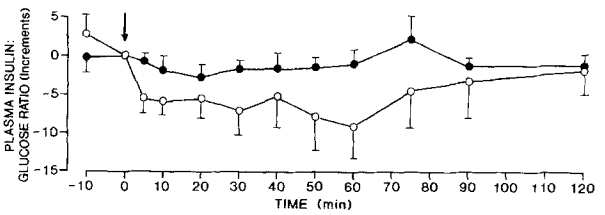

Figure 3. Changes in mean arterial plasma insulin: glucose ratio in response to L-NAME (100 μmol kg^{-1} i.a.; ↓) followed by sodium nitroprusside (4–8 nmol min^{-1} kg^{-1} i.v.) in 6 normal control calves (●) and in 8 calves given exogenous glucose (30–60 μmol min^{-1} kg^{-1} i.v.; ○).

also produced a fall in the mean arterial plasma concentration of pancreatic glucagon and PP in the absence of exogenous glucose, but neither fall achieved statistical significance (data not shown).

These results show that when plasma glucose concentration is artificially raised (by about 50%) and nitric oxide synthase is then inhibited by L-NAME the effect of glucose on plasma insulin concentration is somehow reduced. The most likely explanation for this phenomenon is that the insulin response to hyperglycaemia is mediated, at least in part, by a nitric oxide-related agonist. The finding that the concentration of insulin in the arterial plasma was persistently significantly reduced in the face of a significant rise in plasma glucose, which would intensify the stimulus to release it from the β-cells, testifies to the potency of the effect. In the conscious calf the insulin response to hyperglycaemia is mediated largely via the vagal parasympathetic innervation[8] raising the possibility that NOx is released from postganglionic nerve terminals to act directly on the β-cells. However, the insulin response to direct electrical stimulation of the vagus nerves themselves is abolished by atropine[9] indicating that NOx release is more likely to depend on muscarinic activation of some other site of release such as endothelial cells. The present study was not designed to resolve this question. It is, however, the first report, of which we are aware in which NOx has been implicated in the control of plasma insulin concentration in vivo.

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