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European Journal of Pharmacology 280 (1995) 27–35

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Involvement of nitric oxide in neuroglycopenia-induced insulin and glucagon secretion in the mouse

Bo Ahrén [a,*], Sven Karlsson [a], Anton J.W. Scheurink [b], Anton B. Steffens [b]

[a] *Department of Medicine, Lund University, Malmö, Sweden*
[b] *Department of Animal Physiology, University of Groningen, Haren, Netherlands*

Received 21 November 1994; revised 7 March 1995; accepted 14 March 1995

Abstract

Neuroglycopenia induced by administration of 2-deoxy-D-glucose is known to stimulate the secretion of both insulin and glucagon in mice by a mechanism that is dependent on neural activity. In the present study, we examined whether the neurotransmitter nitric oxide (NO) is involved in this process. Therefore, 2-deoxy-D-glucose (500 mg/kg) was injected intravenously alone or together with the inhibitor of NO synthase, N^G-nitro-L-arginine methyl ester (50 mg/kg) to conscious mice. It was found that N^G-nitro-L-arginine methyl ester inhibited the increased plasma levels of both insulin (by 26%; $P = 0.039$) and glucagon (by 45%; $P < 0.001$) at 10 min after injection of 2-deoxy-D-glucose. Similarly, the NO synthase inhibitor, N^G-nitro-L-arginine, which is devoid of the anticholinergic property of N^G-nitro-L-arginine methyl ester, inhibited the responses of both insulin (by 53%; $P = 0.026$) and glucagon (by 57%; $P = 0.003$) to 2-deoxy-D-glucose. In contrast, the stereoisomer of N^G-nitro-L-arginine methyl ester, N^G-nitro-D-arginine methyl ester, which is devoid of NO synthase inhibitory activity, was without effect on 2-deoxy-D-glucose-induced insulin and glucagon secretion. Plasma levels of adrenaline and noradrenaline after administration of 2-deoxy-D-glucose were also reduced by N^G-nitro-L-arginine methyl ester. In contrast, the insulin and glucagon secretory responses to intravenous injection of arginine (250 mg/kg), glucose (500 mg/kg) or the cholinergic agonist, carbachol (30 μg/kg), were not influenced by N^G-nitro-L-arginine methyl ester, N^G-nitro-D-arginine methyl ester or N^G-nitro-L-arginine. We conclude that the increased secretion of both insulin and glucagon during neuroglycopenia in the mouse is partially mediated by NO.

Keywords: Insulin secretion; Glucagon secretion; Neuroglycopenia; 2-Deoxy-D-glucose; Nitric oxide (NO); Nitric oxide synthase; N^G-Nitro-L-arginine methyl ester; N^G-Nitro-L-arginine; (Mouse)

1. Introduction

Nitric oxide (NO) was initially described as the endothelium-derived relaxing factor in blood vessels (Palmer et al., 1987), and later studies revealed that the gas is an intracellular messenger in a variety of cells (see Moncada et al., 1991; Stark and Szurszewski, 1992). In addition, NO has been suggested to function as a neurotransmitter, since the enzyme that catalyzes the formation of NO, NO synthase, has been localized to central as well as peripheral nerves (Bredt et al., 1990), since NO is released from nerves during nerve activation (Bult et al., 1990) and since blockade of NO formation inhibits neurally evoked effects (Allescher et al., 1992; Sanders and Ward, 1992).

As a neurotransmitter, NO may be involved in neurally regulated metabolic processes. One such important neural process is the metabolic defense against neuroglycopenia or hypoglycemia. Thus, when the ambient plasma glucose level drops or when central glycopenia evolves, the autonomic nervous system is activated in order to counteract the glucose deprivation by means of increased secretion of glucagon and adrenaline (see Havel and Taborsky, 1989). The mechanisms underlying the activation of these counter-regulatory responses are not established. However, the mechanisms are of great importance to delineate, since in diabetes, the hypoglycemia-induced glucagon secretion is impaired (Benson et al., 1977; Patel, 1983).

* Corresponding author. Department of Medicine, Lund University, Malmö General Hospital, S-214 01 Malmö, Sweden.

SSDI 0014-2999(95)00171-9

Whether NO is involved in the mediation of these processes was the subject of the present study.

We have previously demonstrated that neuroglycopenia induced by the glucose analogue, 2-deoxy-D-glucose, stimulates the secretion of both insulin and glucagon in the mouse and that this effect is largely attributable to neural responses since it is inhibited by both the ganglionic inhibitor hexamethonium and the muscarinic receptor antagonist methylatropine (Karlsson and Ahrén, 1987; Karlsson et al., 1987). In the present study, we have examined whether inhibition of NO synthase affects the insulin and glucagon responses to 2-deoxy-D-glucose in mice. We inhibited the activity of NO synthase by means of the arginine analogues, N^G-nitro-L-arginine methyl ester and N^G-nitro-L-arginine (Lambert et al., 1991). As a control, the stereoisomer of N^G-nitro-L-arginine methyl ester, N^G-nitro-D-arginine methyl ester, which is devoid of NO synthase inhibitory action (Peterson et al., 1992), was used, and, furthermore, to control for direct A and B cell actions of the drugs used, controls given arginine, glucose and the cholinergic agonist, carbachol, were also included.

2. Materials and methods

2.1. Animals

Female mice of the NMRI strain (Bomholtgård Breeding and Research Centre, Ry, Denmark), weighing 25–30 g, were used. The animals were kept on a standard pellet diet and tap water ad libitum.

2.2. Drugs

D-Glucose, L-arginine hydrochloride, carbachol (= carbamoylcholine chloride), N^G-nitro-L-arginine methyl ester, N^G-nitro-D-arginine methyl ester, N^G-nitro-L-arginine and 2-deoxy-D-glucose were all from Sigma Chemical Co. (St. Louis, MO, USA). The compounds were dissolved in 0.9% saline.

2.3. Experimental protocol

In the first experimental series, 2-deoxy-D-glucose (500 mg/kg = 3.0 mmol/kg) was injected intravenously into a tail vein alone or together with N^G-nitro-L-arginine methyl ester (50 mg/kg = 0.20 mmol/kg), N^G-nitro-D-arginine methyl ester (50 mg/kg = 0.20 mmol/kg) or N^G-nitro-L-arginine (50 mg/kg = 0.23 mmol/kg). Controls were injected with saline alone or in combination with N^G-nitro-L-arginine methyl ester, N^G-nitro-D-arginine methyl ester or N^G-nitro-L-arginine. The volume load was 10 μl/g body weight. Blood samples were taken from the retroorbital plexus at either 2 or at 10 min after the injection. These time points were selected since we have previously shown that plasma levels of both insulin and glucagon are markedly elevated at these time points following intravenous injection of 2-deoxy-D-glucose (Karlsson and Ahrén, 1987; Karlsson et al., 1987). In the second experimental series, D-glucose (500 mg/kg = 2.8 mmol/kg), L-arginine (250 mg/kg = 1.2 mmol/kg) or carbachol (30 μg/kg = 0.16 μmol/kg) was injected intravenously into a tail vein alone or together with N^G-nitro-L-arginine methyl ester (50 mg/kg = 0.20 mmol/kg), N^G-nitro-D-arginine methyl ester (50 mg/kg = 0.20 mmol/kg) or N^G-nitro-L-arginine (50 mg/kg = 0.23 mmol/kg). Controls were given saline alone or were injected with N^G-nitro-L-arginine methyl ester, N^G-nitro-D-arginine methyl ester or N^G-nitro-L-arginine. The volume load was 10 μl/g body weight. Blood samples were taken from the retroorbital plexus at 2 min after the injection, since at this time point plasma levels of insulin and glucagon are maximal after injection of these secretagogues (Ahrén and Lundquist, 1981a,1982,1986). The blood samples for glucose and insulin were taken in heparinized tubes, the blood samples for glucagon were taken in chilled (0° C) tubes containing aprotinine, whereas blood samples for catecholamines were taken in chilled tubes containing heparin and EDTA. Following centrifugation at 4° C, plasma was decanted and frozen at −20° C or at −80° C until assayed.

2.4. Assays

Plasma insulin was determined by radioimmunoassay using a guinea pig anti-rat insulin antibody (Linco Res, St. Louis, MO, USA), ^{125}I-labelled porcine insulin (Novo Nordic, Bagsvaerd, Denmark) as tracer and rat insulin (Linco) as standard. The bound antigen-antibody complex was precipitated by the use of an anti-IgG (goat anti-guinea pig) antibody (Linco). Plasma glucagon was determined by radioimmunoassay from small samples (25 μl) of unextracted plasma as previously described (Ahrén and Lundquist, 1982). Plasma glucose levels were determined by the glucose oxidase method. Plasma levels of adrenaline and noradrenaline were determined by liquid chromatography in combination with electrochemical detection as previously described (Scheurink and Ritter, 1993).

2.5. Statistics

The results are expressed as means ± S.E.M. Analysis of variance followed by Newman-Keuls post-hoc test with Bonferroni correction for multiple comparisons or Student's t-test was used for statistical evaluation.

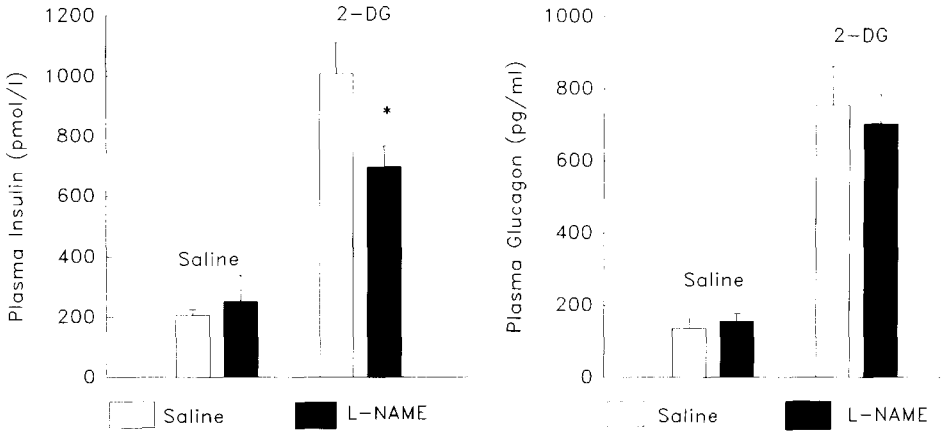

Fig. 1. Plasma levels of insulin (left panel) and glucagon (right panel) at 2 min after an intravenous injection of 2-deoxy-D-glucose (500 mg/kg) alone or together with N^G-nitro-L-arginine methyl ester (L-NAME in figure; 50 mg/kg) in mice. Controls were injected with saline. There were 23 or 24 animals in each group. Means ± S.E.M. are shown. Asterisk indicates a probability level of random difference between groups of * $P < 0.05$.

3. Results

3.1. Effects of N^G-nitro-L-arginine methyl ester on 2-deoxy-D-glucose-stimulated insulin and glucagon secretion and plasma glucose levels

The intravenous injection of 2-deoxy-D-glucose markedly increased plasma insulin and glucagon when compared to the saline-injected controls, both at 2 min (Fig. 1) and at 10 min (Fig. 2) after the injection of 2-deoxy-D-glucose. Simultaneous injection of N^G-nitro-L-arginine methyl ester inhibited the 2-deoxy-D-glucose-induced increase in plasma insulin levels both at 2 min (by 44%; $P = 0.015$) and at 10 min (by 26%; $P = 0.003$), whereas the 2-deoxy-D-glucose-induced increase in plasma glucagon levels was significantly reduced by N^G-nitro-L-arginine methyl ester at 10 min (by 45%; $P < 0.001$) but not at 2 min after injection of 2-deoxy-D-glucose. Plasma glucose levels were significantly reduced by N^G-nitro-L-arginine methyl ester at 10 min after injection, both in controls and in 2-deoxy-D-glucose-injected animals, but not at 2 min after injection (Table 1).

3.2. Effects of N^G-nitro-L-arginine methyl ester on plasma catecholamines after 2-deoxy-D-glucose

Plasma levels of adrenaline at both 2 and 10 min after injection of 2-deoxy-D-glucose as well as in controls were reduced by N^G-nitro-L-arginine methyl ester (Table 2). Plasma levels of noradrenaline were significantly reduced by N^G-nitro-L-arginine methyl ester at 10 min after injection (Table 2; $P < 0.001$). In contrast, at 2 min after injection the difference in plasma nor-

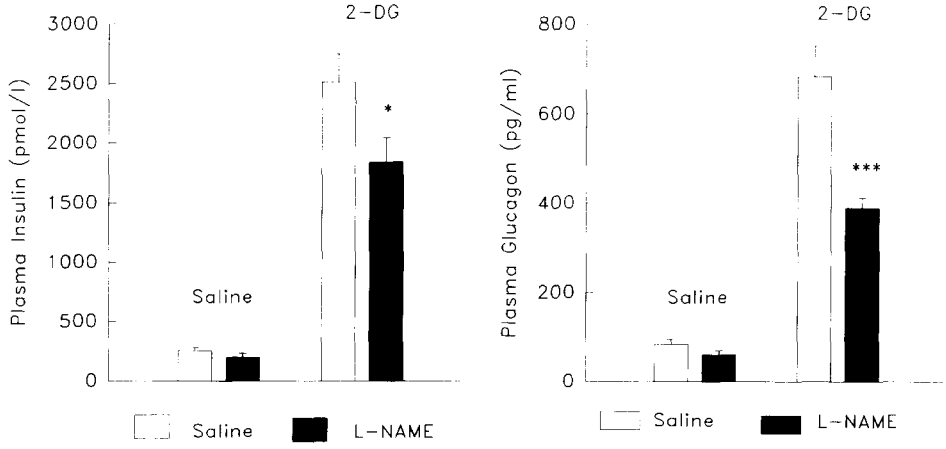

Fig. 2. Plasma levels of insulin (left panel) and glucagon (right panel) at 10 min after an intravenous injection of 2-deoxy-D-glucose (500 mg/kg) alone or together with N^G-nitro-L-arginine methyl ester (L-NAME in figure; 50 mg/kg) in mice. Controls were injected with saline. There were 29 or 30 animals in each group. Means ± S.E.M. are shown. Asterisks indicate a probability level of random difference between groups: * $P < 0.05$; *** $P < 0.001$.

Table 1
Plasma glucose levels at 2 or 10 min after an intravenous injection of 2-deoxy-D-glucose (500 mg/kg) alone or together with N^G-nitro-L-arginine methyl ester (50 mg/kg) in mice

	Saline	2-Deoxy-D-glucose
(A) 2 min		
Saline	8.7 ± 0.2 mmol/l (n = 24)	13.8 ± 0.3 mmol/l (n = 24)
N^G-Nitro-L-arginine methyl ester	8.7 ± 0.2 mmol/l (n = 23)	14.0 ± 0.2 mmol/l (n = 24)
(B) 10 min		
Saline	8.7 ± 0.3 mmol/l (n = 29)	15.8 ± 0.3 mmol/l (n = 30)
N^G-Nitro-L-arginine methyl ester	7.6 ± 0.2 mmol/l (n = 29) [a]	13.6 ± 0.4 mmol/l (n = 30) [b]

Controls were given saline. Means ± S.E.M. are shown. 'n' indicates number of animals. [a] $P = 0.003$ vs. saline-saline group; [b] $P < 0.001$ vs. saline-saline group.

Table 2
Plasma adrenaline and noradrenaline levels at 2 or 10 min after an intravenous injection of 2-deoxy-D-glucose (2-DG; 500 mg/kg) alone or together with N^G-nitro-L-arginine methyl ester (50 mg/kg) in mice

	Saline	2-DG 2 min	2-DG 10 min
Adrenaline			
Saline	5.0 ± 0.8 ng/ml (n = 15)	7.8 ± 0.8 ng/ml (n = 16)	7.9 ± 0.8 ng/ml (n = 20)
N^G-Nitro-L-arginine methyl ester	2.3 ± 0.3 ng/ml (n = 16) [b]	5.2 ± 0.6 ng/ml (n = 16) [a]	3.9 ± 0.4 ng/ml (n = 20) [b]
Noradrenaline			
Saline	4.3 ± 0.6 ng/ml (n = 15)	4.6 ± 0.5 ng/ml (n = 16)	4.4 ± 0.6 ng/ml (n = 20)
N^G-Nitro-L-arginine methyl ester	2.8 ± 0.4 ng/ml (n = 16) [b]	3.3 ± 0.5 ng/ml (n = 16) (P = 0.080)	2.4 ± 0.2 ng/ml (n = 20) [b]

Controls were given saline (the 2 min samples are shown in the Table). Means ± S.E.M. are shown. 'n' indicates number of animals. [a] $P = 0.012$ vs. the control group; [b] $P < 0.001$ vs. the control group.

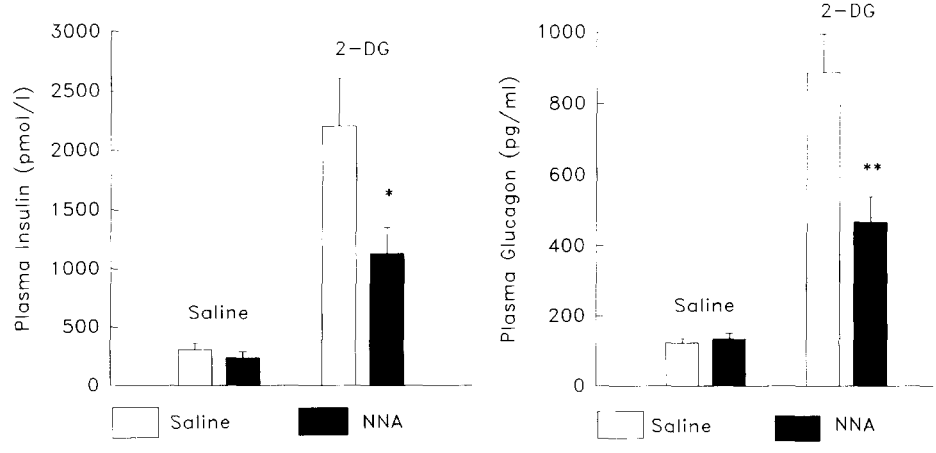

Fig. 3. Plasma levels of insulin (left panel) and glucagon (right panel) at 10 min after the intravenous injection of 2-deoxy-D-glucose (500 mg/kg) alone or together with N^G-nitro-L-arginine (NNA in figure; 50 mg/kg) in mice. Controls were injected with saline. There were 15 or 16 animals in each group. Means ± S.E.M. are shown. Asterisks indicate a probability level of random difference between groups: * $P < 0.05$; ** $P < 0.01$.

Table 3
Plasma glucose levels at 10 min after an intravenous injection of 2-deoxy-D-glucose (500 mg/kg) alone or together with N^G-nitro-L-arginine (50 mg/kg) in mice

	Saline	2-Deoxy-D-glucose
Saline	8.6 ± 0.2 mmol/l (n = 16)	17.5 ± 0.4 mmol/l (n = 16)
N^G-Nitro-L-arginine	9.2 ± 0.4 mmol/l (n = 15)	15.9 ± 0.5 mmol/l (n = 16) [a]

Controls were given saline. Means ± S.E.M. are shown. 'n' indicates number of animals. [a] P = 0.026 v.s saline-saline groups.

adrenaline between 2-deoxy-D-glucose- and 2-deoxy-D-glucose + N^G-nitro-L-arginine methyl ester-injected mice did not reach significance (Table 2; P = 0.080).

3.3. Effects of N^G-nitro-D-arginine methyl ester on 2-deoxy-D-glucose-stimulated insulin and glucagon secretion and plasma glucose levels

In this experimental series, plasma insulin levels were 405 ± 74 pmol/l in saline-injected animals (n = 16) and 1085 ± 148 pmol/l in 2-deoxy-D-glucose-injected animals (n = 15; $P < 0.001$) at 2 min after injection. N^G-Nitro-D-arginine methyl ester did not affect plasma insulin levels, being 414 ± 73 pmol/l in the saline-injected animals and 946 ± 89 pmol/l (n = 16) in 2-deoxy-D-glucose-injected animals (n = 16). At 10 min after injection, plasma insulin levels were 404 ± 58 pmol/l in saline-injected controls (n = 16) and 2204 ± 404 pmol/l after 2-deoxy-D-glucose (n = 16), and these figures were not significantly affected by N^G-nitro-D-arginine methyl ester, being 396 ± 64 pmol/l (n = 16) and 2247 ± 446 pmol/l (n = 16), respectively (n.s.). Plasma glucagon levels at 2 min after injection were 100 ± 9 pg/ml in controls (n = 16) and 699 ± 80 pg/ml after 2-deoxy-D-glucose (n = 15; $P < 0.001$) and at 10 min, they were 122 ± 13 pg/ml in controls (n = 16) and 888 ± 106 pg/ml after 2-deoxy-D-glucose (n = 16; $P < 0.001$). N^G-Nitro-D-arginine methyl ester did not affect the plasma glucagon levels, neither at 2 min (being 121 ± 23 pg/ml in controls and 734 ± 93 pg/ml after 2-deoxy-D-glucose) nor at 10 min (being 98 ± 12 pg/ml in controls and 910 ± 93 pg/ml after 2-deoxy-D-glucose). Similarly, plasma glucose levels were not significantly affected by N^G-nitro-D-arginine methyl ester under any of these conditions (data not shown).

3.4. Effects of N^G-nitro-L-arginine on 2-deoxy-D-glucose-stimulated insulin and glucagon secretion and plasma glucose levels

The intravenous injection of N^G-nitro-L-arginine significantly reduced the 2-deoxy-D-glucose-stimulated increase in plasma levels of insulin (by 53%; P = 0.026) and glucagon (by 57%; P = 0.003) in samples taken at 10 min after injection (Fig. 3), and the plasma glucose levels after 2-deoxy-D-glucose were slightly reduced by N^G-nitro-L-arginine (Table 3).

3.5. Effects of N^G-nitro-L-arginine methyl ester and N^G-nitro-D-arginine methyl ester on plasma insulin, glucagon and glucose after arginine and carbachol

The intravenous injection of arginine increased plasma levels of insulin and glucagon after 2 min ($P < 0.001$). Simultaneous injection of N^G-nitro-L-arginine methyl ester (Fig. 4) or N^G-nitro-D-arginine methyl ester (data not shown; n = 15–16 in each group) did not affect these increases. Similarly, the intravenous injection of glucose increased plasma levels of insulin at 2 min ($P < 0.001$), and this increase was not

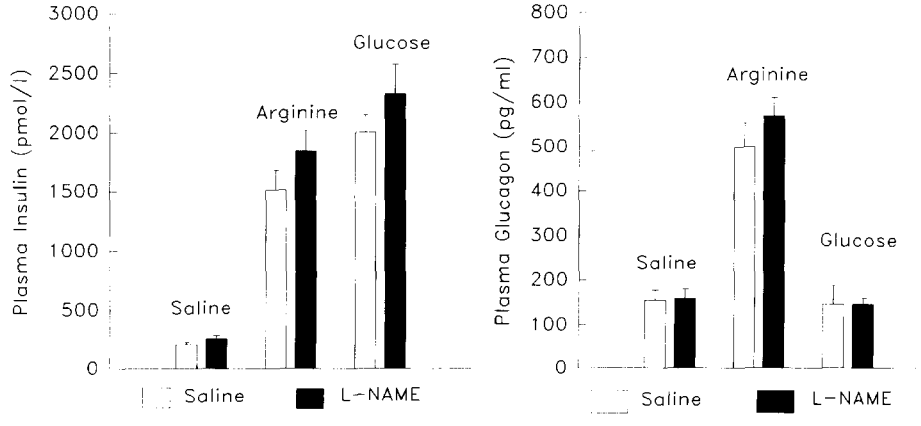

Fig. 4. Plasma levels of insulin (left panel) and glucagon (right panel) at 2 min after the intravenous injection of arginine (250 mg/kg) or glucose (500 mg/kg) alone or together with N^G-nitro-L-arginine methyl ester (L-NAME in figure; 50 mg/kg) in mice. Controls were injected with saline. There were 28–31 animals in each group. Means ± S.E.M. are shown.

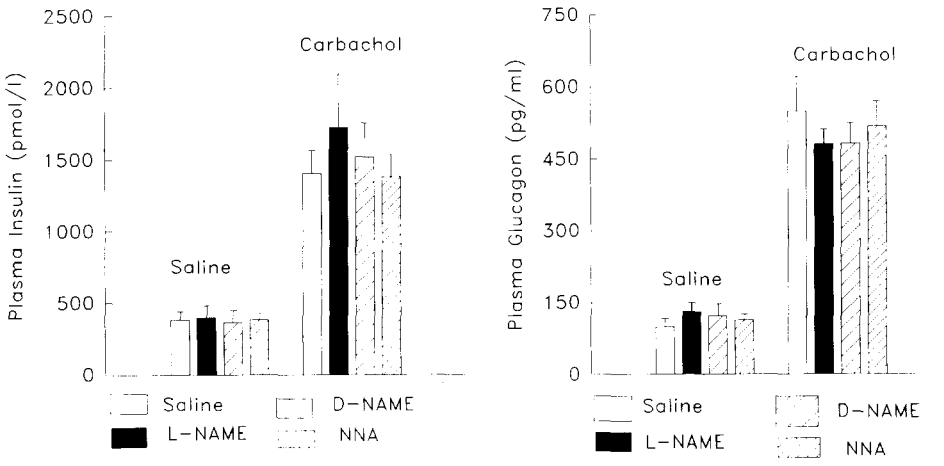

Fig. 5. Plasma levels of insulin (left panel) and glucagon (right panel) at 2 min after the intravenous injection of carbachol (30 μg/kg) alone or together with N^G-nitro-L-arginine methyl ester (L-NAME in figure; 50 mg/kg), N^G-nitro-D-arginine methyl ester (D-NAME in figure; 50 mg/kg) or N^G-nitro-L-arginine (NNA in figure; 50 mg/kg) in mice. Controls were injected with saline. There were 14–16 animals in each group. Means ± S.E.M. are shown.

significantly affected by N^G-nitro-L-arginine methyl ester (Fig. 4) or N^G-nitro-D-arginine methyl ester (data not shown; $n = 15–16$ animals in each group). Plasma glucose levels were not significantly affected by N^G-nitro-L-arginine methyl ester or N^G-nitro-D-arginine methyl ester under these conditions.

3.6. Effects of N^G-nitro-L-arginine methyl ester, N^G-nitro-D-arginine methyl ester or N^G-nitro-L-arginine on carbachol-stimulated insulin and glucagon secretion and plasma glucose levels

The intravenous injection of the cholinergic agonist, carbachol, increased plasma insulin and glucagon after 2 min ($P < 0.001$). Neither N^G-nitro-L-arginine methyl ester, nor N^G-nitro-D-arginine methyl ester or N^G-nitro-L-arginine did significantly affect these increases (Fig. 5), nor did the drugs affect plasma glucose levels (data not shown).

4. Discussion

Nitric oxide is synthesized when L-arginine is converted to NO and L-citrulline by NO synthase (see Eizirik and Leijerstam, 1994). NO synthase occurs in two different forms, of which one is a calcium-sensitive, constitutive, form, occurring in endothelial cells and in nerves, whereas the other form is a calcium-insensitive, inducible, form, which occurs in macrophages (see Radomski et al., 1991; Eizirik and Leijerstam, 1994). A major effect of NO is to stimulate guanylate cyclase activity in target cells and thereby increase the cellular content of cyclic guanosine 3′5′-monophosphate (cyclic GMP; see Ignarro, 1989). It has previously been speculated whether NO formed through the constitutive NO synthase is involved in the normal regulation of insulin secretion, since glucose- and arginine-stimulated insulin secretion has been shown to be inhibited by NO synthase inhibition both under in vitro conditions (Laychock et al., 1991; Schmidt et al., 1992) and in vivo in rats (Schmidt et al., 1992). This is, however, controversial, since it has also been shown that in isolated islets, the insulin secretagogues, glucose, arginine and carbachol, do not stimulate the synthesis of NO or the accumulation of cyclic GMP and that NO synthase inhibition does not affect secretagogue-stimulated insulin secretion (Southern et al., 1990; Jones et al., 1992). Furthermore, in the perfused rat pancreas, NO synthase inhibition does not affect glucose-stimulated insulin secretion (Weigert et al., 1992) and in a study in rats treated on a long-term basis (4 weeks) with NO synthase inhibition, insulin secretion was not affected (Pueyo et al., 1994). We show in the present study that the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester, does not inhibit carbachol-, arginine- or glucose-stimulated insulin secretion or arginine-stimulated glucagon secretion in the mouse. This result therefore supports the studies which suggest that islet NO is of no importance for the normal regulation of islet hormone secretion.

The non-metabolized glucose analogue, 2-deoxy-D-glucose, competes with glucose for membrane transport and for intracellular phosphorylation (Wick et al., 1957; Brown, 1962). 2-Deoxy-D-glucose thereby blocks the glucose metabolism at the glycolytic level and creates intracellular glycopenia. This induces neuroglycopenia, since the central nervous system exhibits a higher sensitivity to glucose deprivation (Müller et al., 1971). The neuroglycopenia in turn activates the autonomic nervous system (see Havel and Taborsky, 1989) with a resulting rise in blood glucose levels. In the

mouse, this activation of the autonomic nervous system by 2-deoxy-D-glucose induces secretion of both insulin and glucagon (Karlsson and Ahrén, 1987; Karlsson et al., 1987). In the present study, we found that both N^G-nitro-L-arginine methyl ester and N^G-nitro-L-arginine by approximately 40% inhibited both these responses to 2-deoxy-D-glucose with an accompanying reduction in the 2-deoxy-D-glucose-induced increase in plasma glucose. Therefore, we conclude that the neuroglycopenia-induced islet hormone secretion is partially mediated by the NO pathway.

We have previously shown that the islet hormone responses to 2-deoxy-D-glucose in mice to a large degree is dependent on cholinergic integrity, since methylatropine abolishes the responses (Karlsson and Ahrén, 1987; Karlsson et al., 1987). We do not, however, consider the findings in the present study to be caused by an anticholinergic property of N^G-nitro-L-arginine methyl ester, even though such an action has been described in other experimental systems (Buxton et al., 1993). Thus, first, N^G-nitro-L-arginine methyl ester did not affect carbachol-stimulated insulin and glucagon secretion, and, second, N^G-nitro-L-arginine, which is devoid of anticholinergic property (Buxton et al., 1993), inhibited 2-deoxy-D-glucose-induced insulin and glucagon secretion to the same extent as did N^G-nitro-L-arginine methyl ester. Furthermore, it has been shown that N^G-nitro-L-arginine methyl ester inhibits the reduction of ferric cytochrome *C* induced by both ferrous iron and adrenaline, which could imply that the drug affects multiple biological systems (Peterson et al., 1992). However, we do not consider the results of the present study to be dependent on non-specific, NO synthase-independent, actions of N^G-nitro-L-arginine methyl ester, since N^G-nitro-D-arginine methyl ester, which is devoid of NO synthase inhibition but shares other properties of N^G-nitro-L-arginine methyl ester (Peterson et al., 1992), did not affect 2-deoxy-D-glucose-induced insulin and glucagon secretion. Taken together, our results instead indicate that 2-deoxy-D-glucose-induced insulin and glucagon secretion are dependent on NO.

At present we do not know which level in the chain of events initiated by 2-deoxy-D-glucose that involves NO. Central neurons might be involved, since central NO neurons have been described by immunocytochemistry (Bredt et al., 1990), since NO synthase mRNA has been localized to the hypothalamus (Grossman et al., 1994) and since NO synthase has been pharmacologically described in the dorsal motor nucleus of the vagus (Travagli and Gillis, 1994). Also peripheral sites might be implicated, however, since, for example, NO containing neurons have been described within the pancreas (Vincent, 1992) and vagally induced pancreatic exocrine secretion in the pig pancreas is inhibited by NO synthase inhibition (Holst et al., 1994). Similarly, cholinergically induced neurogenic vasodilatation has been shown to be mediated by NO in the dog hindlimb (Loke et al., 1994). An interesting recent observation in this context is the inhibition by N^G-nitro-L-arginine methyl ester of glucose-stimulated insulin secretion in calves (Edwards et al., 1994), since in this species, glucose-stimulated insulin secretion seems to be a process which is dependent on the integrity of parasympathetic nerves (Bloom and Edwards, 1981). Such a mechanism is, however, unlikely to account for the inhibition of N^G-nitro-L-arginine methyl ester on 2-deoxy-D-glucose-induced insulin secretion in the mouse, since we have previously shown that glucose-stimulated insulin secretion is not affected by muscarinic receptor blockade in this species (Ahrén and Lundquist, 1981b). Taken together, therefore, we conclude that neuroglycopenia-induced islet hormone secretion is partially mediated by NO, although we cannot identify the level for this dependence.

It is known that 2-deoxy-D-glucose increases plasma levels of adrenaline and noradrenaline (Scheurink and Ritter, 1993). In the present study, we show that N^G-nitro-L-arginine methyl ester reduces the plasma levels of adrenaline and noradrenaline, both under basal conditions and after 2-deoxy-D-glucose. This suggests that N^G-nitro-L-arginine methyl ester inhibits the release both of adrenaline from the adrenals and of noradrenaline from sympathetic nerve terminals. These conclusions are consistent with previous findings that NO synthase is present in the adrenal glands (Palacio et al., 1989), that the adrenals are innervated by NO synthase containing nerves (Bredt et al., 1990), that NO stimulates adrenaline secretion from adrenal medulla (Dohi et al., 1983), and that NO synthase inhibition inhibits neurotransmitter release from sympathetic hypogastric nerves (Thatikunta et al., 1993). It is, however, unlikely that these effects mediate the impaired insulin response to 2-deoxy-D-glucose, since we have previously shown that adrenalectomy potentiates 2-deoxy-D-glucose-induced insulin secretion in the mouse (Karlsson and Ahrén, 1991) and it is known that adrenaline and noradrenaline both inhibit stimulated insulin secretion (see Ahrén et al., 1986). Therefore, if anything, the reduced plasma levels of catecholamines would tend to increase, rather than to reduce, the 2-deoxy-D-glucose-stimulated insulin secretion. On the other hand, regarding glucagon secretion, both adrenalectomy and chemical sympathectomy by means of 6-hydroxydopamine partially inhibit 2-deoxy-D-glucose-stimulated glucagon secretion in the mouse (Karlsson and Ahrén, 1991), which could imply that the reduced plasma catecholamines following N^G-nitro-L-arginine methyl ester would contribute to the accompanying impaired 2-deoxy-D-glucose-stimulated glucagon secretion.

An intriguing observation in the present paper is

that the glucagon response to 2-deoxy-D-glucose was inhibited by N^G-nitro-L-arginine methyl ester at 10 min but not at 2 min after 2-deoxy-D-glucose administration. This implies that the glucagon response to neuroglycopenia may be divided into two different phases with different underlying mechanisms: an early, NO-independent phase, and a later, NO-dependent phase. Interestingly, the sensitivity also to methylatropine for the 2-deoxy-D-glucose-stimulated glucagon secretion is different at 2 vs. at 10 min after injection, since the glucagon response is totally abolished by methylatropine at 10 min (Karlsson and Ahrén, 1987) but only partially inhibited at 2 min (Karlsson and Ahrén, 1990), yet the insulin response is totally abolished at both time points (Karlsson and Ahrén, 1990; Karlsson et al., 1987). Altogether, this suggests mediation of 2-deoxy-D-glucose-induced islet hormone secretion by a NO pathway activated by 2-deoxy-D-glucose using parasympathetic nerves with acetylcholine as the distal branch. We also observed in this study that N^G-nitro-L-arginine methyl ester slightly reduced plasma glucose levels at 10 min after administration. Since this change was not accompanied by any alterations in basal levels of plasma insulin or glucagon, it was probably due to an action of the NO synthase inhibition on the liver or on muscle or adipose glucose metabolism. A mediating mechanism might be the reduced plasma levels of the glycogenolytic hormone which was induced by N^G-nitro-L-arginine methyl ester. However, the exact mechanism underlying the reduced plasma glucose levels remains to be established.

In conclusion, this study has shown that insulin and glucagon secretion initiated by 2-deoxy-D-glucose-induced neuroglycopenia in the mouse are partially inhibited by the NO synthase inhibiting agents, N^G-nitro-L-arginine methyl ester and N^G-nitro-L-arginine, but not affected by the stereoisomer of N^G-nitro-L-arginine methyl ester, N^G-nitro-D-arginine methyl ester, which is devoid of NO synthase inhibiting action. Furthermore, the study has shown that NO synthase inhibition does not affect plasma insulin or glucagon after stimulation with glucose, arginine or the cholinergic agonist, carbachol. The study therefore suggests that the neuroglycopenia-induced stimulation of islet hormone secretion is partially dependent on NO.

Acknowledgements

The authors are grateful to Lilian Bengtsson and Lena Kvist for technical assistance, and to the Swedish Medical Research Council (grants Nos. 14 × -6834 and 12 × -712), Albert Påhlsson, Crafoord, Novo Nordisk and Lundström Foundations, the Swedish Diabetes Association and the Faculty of Medicine, Lund University for financial assistance.

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