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L-arginine and pancreatic islet blood flow in anesthetized rats

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Abstract. The aim of the present study was to see if L-arginine, which induces insulin release and is a precursor of the endothelial-derived relaxing factor nitric oxide, affects whole pancreatic and/or islet blood flow. For this purpose, anesthetized male Sprague-Dawley rats were injected intravenously with either saline or L-arginine (25, 100 or 250 mg/kg body weight). All doses of arginine caused a slight increase in blood glucose concentration, while the highest dose (250 mg/kg body weight) also increased insulin concentration. However, no changes in either mean arterial blood pressure, whole pancreatic or islet blood flow could be discerned with any of the doses of arginine used. It is concluded that insulin release is not necessarily associated with an increased islet blood perfusion.
Key words. Pancreatic islets; islet blood flow; arginine; insulin secretion.

The capillary system of the pancreatic islets consists of sinusoidal blood vessels, around which the endocrine cells are arranged in a polar fashion[1, 2]. It is known that both nervous[3], hormonal[4] and local factors[5] can selectively affect islet blood flow. The independent regulation of islet blood perfusion is likely to be due to effects on vascular smooth muscle within afferent islet blood vessels. To what extent the different response of arterioles supplying the endocrine and exocrine pancreas is due to differences in the vascular smooth muscle per se, to differences in innervation or in the production of local endothelial vasoactive substances related to islet metabolism, is presently unknown.

The aim of the present study was to investigate to what extent L-arginine affects islet blood flow. Arginine stimulates insulin release by a direct effect on ionic channels in the plasma membrane[6]. Furthermore, it is a substrate for nitric oxide synthase in the formation of nitric oxide, the main endothelial-derived relaxing factor[7, 8]. Nitric oxide is known to play an important role as a regulator of the general vascular tonus within the body[9] and also of islet blood flow[5].

Materials and methods

Animals. Male Sprague-Dawley rats aged 10–12 weeks from a local breeding colony (Biomedical Center, Uppsala, Sweden) with free access to pelleted food and tap water, were used in all experiments.

Blow flow measurements. This procedure has been described in detail elsewhere[10]. Briefly, the rats were anesthetized with an intraperitoneal injection of pentobarital sodium (60 mg/kg body weight; Mebumal vet®; Nordvacc AB, Solna, Sweden), heparinized, and placed on an operating table maintained at body temperature (38 °C). Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the femoral artery. The aortic catheter was connected to a pressure transducer (PDCR 75/1; Druck Ltd, Groby, UK) to allow continuous monitoring of the mean arterial blood pressure. When the blood pressure had remained stable for at least 20 min, 2 ml/kg body weight of either saline alone or L-arginine (25, 100 or 250 mg/kg body weight) dissolved in saline was injected intravenously. Ten minutes later $1.5–2.0 \times 10^5$ nonradioactive microspheres (NEN-Trac®; DuPont Pharmaceuticals Inc, Wilmington, DE, USA), with a diameter of 11 μm, were injected over a period of 10 s via the catheter with its tip in the ascending aorta. Starting 5 s before the microsphere injection, and continuing for a total of 60 s, an arterial blood sample was collected by free flow from the catheter in the femoral artery at a rate of approximately 0.50 ml/min. The exact withdrawal rate was confirmed in each experiment by weighing the sample. The animals were then killed and the pancreas and adrenal glands were removed, blotted, weighed and treated with a freeze-thawing technique to visualize the pancreatic islets and the microspheres[11]. The microspheres in the adrenal glands, the whole pancreas and the islets were then counted. The number of microspheres in the arterial reference sample was determined by transferring the blood to glass microfibre filters (pore size 0.2 μm), and counting the microspheres in a microscope equipped with transmitted light. The blood flow values were calculated acccording to the formula $Q_{org} = Q_{ref} \times N_{org}/N_{ref}$ where Q_{org} is organ blood flow (ml/min), Q_{ref} is withdrawal rate of reference

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sample (ml/min), N_{org} is number of microspheres present in the organ, and N_{ref} is number of microspheres in the reference sample. Blood flow values based on the microsphere content of the adrenal glands were used to confirm that the microspheres were adequately mixed in the circulation. A difference $< 10\%$ in blood flow values between the glands was taken to indicate adequate mixing.

Glucose and insulin measurements. Blood samples were taken from the femoral catheter immediately before and ten min after administration of saline or arginine. The samples were analyzed for blood glucose concentrations with test reagent strips (ExacTech®; Baxter Travenol Inc, Deerfield, IL, USA). Blood samples for insulin were collected from the catheter in the femoral artery immediately after the blood flow measurements, and later analyzed for insulin content by radioimmunoassay[12].

Statistical calculations. All values are given as means ± SEM. Probabilities of chance differences between the experimental groups were calculated with Student's two-tailed unpaired t-test.

Results

A total of four animals were excluded from the study comprising a total of 34 animals, three due to inadequate mixing of the microspheres with the arterial circulation, and one due to hypotension. Arginine caused a slight increase in blood glucose at all doses given (table 1). Serum insulin concentrations were increased by arginine when given at 250 mg/kg body weight, but not at the lower doses (table 1). No effects on mean arterial blood pressure or whole pancreatic or islet blood flow could be discerned after arginine administration (table 2). Arginine did not influence the fraction of whole pancreatic blood flow diverted through the islet (table 2).

Discussion

The present study shows that high doses of arginine increased both glucose and insulin concentrations in anesthetized rats, thereby confirming previous studies[13]. The increase in blood glucose concentration seen in the

Table 1. Anesthetized Sprague-Dawley rats were injected intravenously (2 ml/kg bw) with saline alone or arginine (25, 100 or 250 mg/kg bw) 10 min before measurements.

Substance given	Saline	Arginine		
		25 mg/kg	100 mg/kg	250 mg/kg
Number of animals	6	8	7	9
Blood glucose concentration (mM)	4.0 ± 0.1	4.8 ± 0.1***	4.5 ± 0.1*	5.9 ± 0.4**
Serum insulin concentration (ng/ml)	0.78 ± 0.21	1.08 ± 0.47	1.25 ± 0.54	4.76 ± 0.70**
Mean arterial blood pressure (mm Hg)	97 ± 6	99 ± 5	96 ± 5	95 ± 4

Values are means ± SEM, * denotes $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ when compared to saline-injected rats.

Table 2. Anesthetized Sprague-Dawley rats were injected intravenously (2 ml/kg bw) with saline alone or arginine (25, 100 or 250 mg/kg bw) 10 min before measurements.

Substance given	Saline	Arginine		
		25 mg/kg	100 mg/kg	250 mg/kg
Number of animals	6	8	7	9
Pancreatic blood flow (ml/min × g pancreas)	0.47 ± 0.07	0.46 ± 0.07	0.43 ± 0.006	0.48 ± 0.05
Islet blood flow (µl/min × g pancreas)	38 ± 7	32 ± 6	39 ± 3	45 ± 5
Islet blood flow (% of whole pancreatic blood flow)	8.2 ± 0.8	6.8 ± 0.6	9.7 ± 0.9	9.8 ± 1.3

Values are means ± SEM.

present study, the mechanisms for which are unknown, was from 4 to approximately 5 mmol/l. Previous investigations have demonstrated that blood glucose in anesthetized rats must be increased to approximately 10 mmol/l, i.e. a much higher level than seen in the present study before any effects on islet blood flow can be discerned[14].

The serum insulin concentration was raised to a similar degree as observed after acute intravenous administration of a bolus dose of glucose by the highest arginine concentration. Glucose induces an islet blood flow increase through nervous mechanisms[3], whereas no effect on islet blood flow was seen after arginine administration. This is somewhat surprising, since it would seem natural that an increased islet metabolism and hormone production would be coupled to an increased blood flow. However, the present findings confim previous studies, where no obligate association between insulin release and islet blood perfusion were found[5, 15, 16]. It seems as if a normal or augmented insulin release can be maintained despite severe reductions in islet blood flow, i.e. the oxygen and nutrients supplied are sufficient to cope with an increased insulin secretion. In view of the high basal islet blood flow and the importance of islet hormones for normal metabolism it is not surprising that the margin of reserve before a functional impairment ensues is considerable. It should be noted that an increased insulin release can actually be associated with a slight decrease in islet blood flow, such as seen after administration of terbutaline[16].

The finding that exogenous administration of L-arginine did not cause any decrease in mean arterial blood pressure suggests that it did not generate nitric oxide in sufficient quantities to affect systemic vascular tone. Furthermore, the lack of specific effects on islet blood flow also suggest that no increase in local production of nitric oxide within the islets occured, since it has been demonstrated that the islets are very sensitive to nitric oxide[5]. In a recent study it was reported that infusion of two times higher doses than those used in the present study caused a slight decrease in blood pressure in rats[17]. However, this hypotension was likely to be induced directly by arginine, and not by associated increase in nitric oxide produciton[17].

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