The glucose dependence of pancreatic endocrine responses to 2-deoxyglucose in the calf[1]

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Summary. The initial plasma glucose concentration of unanesthetized calves with cut splanchnic nerves, given 2-deoxyglucose (1.2 mmoles/kg, i.v.), was either lowered by prior starvation, or raised by a continuous infusion of exogenous glucose. Raising the initial plasma glucose concentration completely suppressed the release of pancreatic glucagon and pancreatic polypeptide but substantially enhanced the release of insulin in response to 2-deoxyglucose.

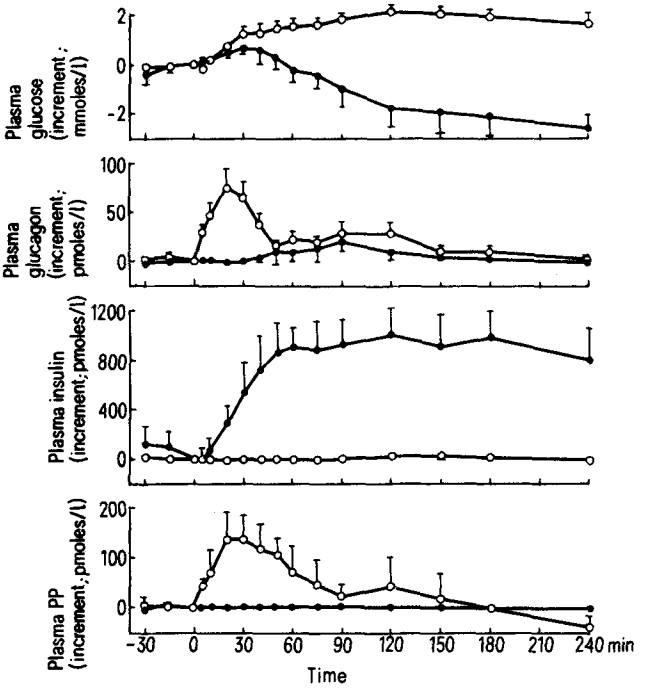
The uptake of glucose by the brain is blocked by 2-deoxyglucose and thus a convenient way is provided of depriving the CNS of glucose without employing exogenous insulin. In unanesthetized calves with cut splanchnic nerves each of the pancreatic endocrine responses to 2-deoxyglucose is mediated exclusively via the parasympathetic innervation to the gland[2-4]. More recently it has been established that the peripheral muscarinic mechanisms that are responsible for the release of both pancreatic glucagon and pancreatic polypeptide (PP) are unaffected by hyperglycaemia[5]. The release of these hormones in response to 2-deoxyglucose has now been examined in unanesthetized calves, with cut splanchnic nerves at different initial glucose concentrations, in order to discover whether the effects of 2-deoxyglucose are reduced in the presence of a high glucose concentration, as would be expected if the blockade of glucose transport across the blood-brains barrier is competitive.

Methods. The experiments were carried out in pedigree Jersey calves in which both splanchnic nerves had been cut and a narrow bore teflon catheter inserted into the saphenous artery under general anesthesia at least 4 days previously. Food but not water was withheld from 1 group of animals for 24–48 h before each experiment in order to reduce the initial plasma glucose concentration. The other group received a priming injection of glucose (0.15 mmoles/kg i.v. in the form of a 1.1 M aqueous solution) 60 min before each experiment, immediately followed by a continuous i.v. infusion of glucose at a rate of 0.05 mmoles $\cdot$ kg^{-1} $\cdot$ min^{-1} for the next 5 h. 2-deoxyglucose (Grade II, Sigma) was administered as a 1.22 M aqueous solution by rapid i.v. injection at a dose of 1.2 mmoles/kg. Samples of arterial blood were collected at intervals into heparinized tubes containing aprotinin (Trasylol, Bayer, 1000 KIU/ml blood) and centrifuged without delay at +4 °C; the plasma was subsequently stored at −20 °C. 2-Deoxyglucose was estimated colorimetrically and glucose by means of a Mark 2 Beckman Glucose Analyzer, making appropriate correction for 2-deoxyglucose where necessary. Pancreatic glucagon, insulin and PP were measured by radioimmunoassays[7-9].

Results and discussion. The injection of 2-deoxyglucose (1.2 mmoles/kg, i.v.) produced a closely similar rise in the concentration of the hexose in the plasma of starved and glucose treated calves, to peak mean values of 3.42±0.21 and 3.55±0.20 mmoles/l respectively, at 5 min. Thereafter the concentration fell exponentially along practically identical time courses in both groups. The absolute mean concentrations of glucose and of the pancreatic hormones in the plasma immediately prior to the administration of 2-deoxyglucose are given in the subscript to the figure, and show that the mean glucagon and PP concentrations were substantially lower and mean insulin concentration substantially higher in the group receiving exogenous glucose.

Raising the mean plasma glucose concentration well above the physiological level (to 12.30±0.47 mmoles/l) completely suppressed the release of both glucagon and PP from the pancreas in response to 2-deoxyglucose (fig.). As the peripheral mechanisms are both insensitive to glucose[5], this finding indicates that 2-deoxyglucose blocks the transport of glucose across the blood-brain barrier less effectively under these conditions and that the intensity of the central stimulus is thus reduced. In spite of this, the release of pancreatic insulin in response to 2-deoxyglucose was significantly potentiated ($p<0.01$) during hyperglycaemia (fig.), providing a striking illustration of the glucose-sensitivity of the peripheral mechanism under conditions when the intensity of the central stimulus was simultaneously reduced. The enhanced insulin output following the administration of 2-deoxyglucose in these experiments proved to be effective in lowering mean plasma glucose concentration in spite of the continued infusion of exogenous glucose (fig.).

These results provide further evidence to support the contention that the reversal of the pancreatic responses of new-born calves to 2-deoxyglucose which occurs after feeding[4], is wholly attributable to the post-prandial rise in plasma glucose concentration.



Comparison of the changes in mean arterial plasma glucose, glucagon, insulin and polypeptide (PP) concentrations in response to 2-deoxyglucose (1.2 mmoles/kg, i.v. at time=0) in conscious 3–5-week-old calves with cut splanchnic nerves, which had either been starved for 24–48 h (○; n=7) or were given exogenous glucose (●; n=6). Vertical bars: SE of each mean value. Absolute values at time=0 in starved calves: glucose, 3.54±0.21 mmoles/l; glucagon, 21±7 pmoles/l; insulin, 17±4 pmoles/l; PP 69±29 pmoles/l. Calves given exogenous glucose: glucose, 12.30±0.47 mmoles/l; glucagon, 3±1 pmoles/l; insulin, 457±205 pmoles/l; PP, 11±3 pmoles/l.

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Formation of coronary arteries sprouting from the primitive aortic sinus wall of the chick embryo

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Summary. The formation of coronary arteries in chick embryos was observed by scanning electron microscopy on injected casts as well as by transmission election microscopy. Usually, 2-4 primitive coronary arteries appear from the right aortic sinus below the level of the cusp margin, and 1-3 from the left one. As development proceeds, the arteries are generally reduced in number to form a single definitive coronary artery on each side. Canalization of the arteries seems to take place by partially degenerative changes of the primordia.

The mechanism of the formation of coronary arteries is considered to be common to many different species of animals, especially mammalian and avian embryos[2]. Evidence is available showing that the coronary arteries first appear as a solid sprout of endothelium from the aortic sinus wall, after the veins are well established. Shortly thereafter, following canalization of the solid sprout, communication with the pre-existing capillaries is established to form the primitive coronary arterial system[3-9]. The present investigation was undertaken to determine whether the number of the coronary arteries initially is one on each side only, and to clarify the sprouting mechanism leading to the formation of coronary arteries from the aortic sinus.

Material and methods. White Leghorn chick embryos ranging from stage 29 to stage 40[10] were used. By the use of vascular casts combined with scanning electron microscopy (SEM), it was possible to obtain detailed information on the microvasculature. To make vascular casts, chick embryos were initially perfused from the heart with physiological saline, followed by injection with Mercox[11]. After the injected resin had hardened, the specimens were placed in a strong solution of KOH to remove the soft tissue. Vascular casts were cleaned, dried, coated with Pt-Pd, and viewed on a JSM-F15 SEM. For transmission electron microscopy (TEM), embryos were perfused with a fixative consisting of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer. Proximal parts of aorta were removed into the same fixative for 2 h at 4 °C. They were post-fixed with 1% OsO_4 for 2 h, dehydrated with ethanol, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead hydroxide, and examined on a JEM-200CX electron microscope.

Results and discussion. Observation of the vascular casts obtained from the embryos up to stage 30 revealed no vascular branches from the aortic sinus on either side, though a part of the capillary network in the cardiac wall is located in the vicinity of the aortic sinuses. At stage 31, primitive coronary areries were found to first arise slightly below the level of the free margin of the excavating aortic cusp. The general view of the cast observed with SEM is shown in figures 1 and 2. 3 primitive coronary arteries sprout from the right aortic sinus and connect with each other (fig. 1). The same pattern is seen on the left (fig. 2). This is confirmed with the light microscopic observation of a cross section of the aortic sinus wall (fig. 4). Figure 3 (stage 35) shows that a single coronary artery arises from the aortic sinus on both the right and left sides. At stages 31-32 (7-7.5 days) the primitive coronary arteries on the right side are from 2 to 4 in number and vary in size, and from 1 to 3 in number on the left side. Concerning this problem, recently Rychter and Ostadal[8], using specimens injected with Indian ink, stated that communication with the aortic cavity doubled symmetrically in the primordia of the coronary arteries in 3 out of 6 cases at 8 days of incubation. However, as far as our investigation is

Figure 1. SEM view of a vascular cast of the right coronary arteries from the chick embryo at stage 31. 3 primitive coronary arteries arise from the aortic sinus (AS) to communicate each other. They are irregular in shape and size. × 300.
Figure 2. SEM view of a cast of the left coronary arteries at stage 31. 3 primitive coronary arteries from the aortic sinus (AS) communicate to from a single descending artery. × 300.
Figure 3. SEM view of a vascular cast of coronary arteries from the chick embryo at stage 35. From both the right and left aortic sinuses (AS), a single coronary artery appears respectively. Shallow pits (arrows) are detected on the cast of the original part of the coronary arteries. RCA, right coronary artery; LCA, left coronary artery. × 60.
Figure 4. A 1 μm cross secrtion of the right aortic sinus at stage 31. 3 primitive coronary arteries (arrows) are detected in the aortic wall. Capillaries (C) which developed in the cardiac wall are located in the vicinity of the arteries. Toluidin blue stain. AL, aortic lumen. × 200.
Figure 5. TEM view of cross section of the aortic sinus from the embryo at stage 31. One of the right primitive coronary arteries is demonstrated. The small lumen is surrounded by irregularly formed primitive endothelial cells with varied electron opacity. AL, aortic lumen. × 1100.
Figure 6. TEM view obtained at stage 30. The solid cell mass arranged vertically to the aortic luminar surface is one of the primordia of the primitive coronary arteries. Large pale cells (PC) are surrrounded by irregularly shaped dark cells with some vacuoles of various sizes. Some of these cells are thought to be degenerative cells because of the obscurity of their organelles and their electron opacity. AL, aortic lumen; C, capillary. × 2400.