SUPPLEMENTARY MATERIAL

Surgical procedures for microsphere measurements

Before going into details on possible errors in the technique we would like to offer a brief overview of the experimental procedures as we perform it in rats. Anesthetized animals are placed on a thermistor controlled heating pad regulated by a rectal probe to maintain the body temperature at 38°C. Arterial catheters are then inserted into the right carotid artery, with its tip being placed in the ascending aorta, and another catheter into a femoral artery. To have a convenient intravenous access we also place a catheter in one of the femoral veins. The latter catheter is used to continuously infuse Ringer (2 ml/kg body weight/hour). After 30-40 min, when the circulatory parameters have stabilized, the microspheres are administered into the carotid catheter as one or two administrations of 300,000 10-µm microspheres dissolved in 0.3 ml saline. Starting 5 sec before each microsphere administration an arterial reference sample is withdrawn from the femoral arterial catheter at a rate of 0.5-0.7 ml/min for 60 sec. This can usually be obtained by free flow. It is important that the flow is not too low or too high, since this affects the precision of the flow measurements (1,2). The arterial reference sample serves as an artificial organ with known flow, and by determining its microsphere contents flow values per g organ weight can be calculated (3). After the experiments arterial blood gases, blood pH, base excess and serum electrolytes are controlled. From the venous catheter we secure samples for plasma glucose and serum insulin determinations. Throughout the procedures one of the arterial catheters is connected to a pressure transducer which allows us to follow arterial blood pressure and heart rate. If central venous pressure is deemed desirable to measure, we insert a separate catheter through the right jugular vein down into the superior vena cava and connect this to another pressure transducer.

Anesthesia: Islet blood flow is very sensitive to ambient glucose concentrations (see below), thereby requiring the use of anesthetics with minor effects on insulin sensitivity and glucose tolerance. We have long experience with thiobutabarbital sodium, which has only minor cardiovascular effects and does not affect glucose tolerance (4). Other barbiturates have negligible effects on glucose tolerance in rodents, and at least the use of pentobarbital gives islet blood flow values similar to those seen in thiobutabarbital-anesthetized animals (4). Some anesthetics, e.g. chloral hydrate and ketamine + xylazine, induce hyperglycemia, thereby markedly affecting islet blood flow (4). Volatile anesthetics such as halothane and isoflurane have only minor effects on blood pressure, but may impair insulin secretion and glucose tolerance (5).

Experiments in awake animals have been performed. In these studies, catheters were implanted 1-2 weeks before the measurements, e.g. (6,7). The great advantage is that anesthetic influences are completely eliminated, and it is easy to demonstrate that no adverse effects of the microsphere injections occur.

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