Pancreatic A and B cell stimulation in euthermic and hibernating marmots (*Marmota flaviventris*): effects of glucose and arginine administration


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Summary. In euthermic and hibernating marmots (*Marmota flaviventris*), the pancreatic A and B cells respond in the appropriate secretory manner to glucose or arginine injection. Although reduced, this response, is clearly present in hibernating marmots. When glucose is administered to euthermic or hibernating marmots, plasma insulin concentrations rise and glucagon levels fall. While similar results are obtained in hibernation, the time period of the response is much longer due to the slowing of temperature dependent metabolic processes. Injection of L-arginine stimulates an increase in plasma glucose, insulin, and glucagon as expected. Measurements of plasma glucose, insulin, and glucagon under basal conditions, suggest that there are no significant differences between any phase of hibernation (e.g. entrance, deep hibernation, arousal) and euthermia. These results provide indirect evidence that the pancreatic A and B cells of hibernating marmots continue to function in order to help regulate plasma glucose concentration.

Introduction

The marmot (*Marmota flaviventris*) is a large sciurid hibernator which has a profound circannual body weight rhythm (Ward and Armitage 1981). Throughout the summer and early fall, marmots spend several hours per day feeding and by late September, virtually all animals attain a peak body weight, cease to feed, and begin to hibernate. During the winter months, regardless of whether the animal is hibernating or not, marmots rely primarily on stored lipid to meet metabolic energy demands (South and House 1967). However, certain tissues, notably the brain and kidney, have a glucose requirement which may be partly fulfilled by ketone bodies (South and House 1967; Rauch and Behrisch 1981). The major gluconeogenic substrate is probably glycerol derived from the degradation of triacylglycerol with minor contributions of substrates from amino acids and perhaps lactate. Glycogenolysis may also contribute to plasma glucose levels, particularly during arousal when muscle energy demands are high (Burlington and Klain 1967; Galster and Morrison 1975).

Although plasma glucose concentration has been measured in many species which hibernate (for a review see, Musacchia and Deavers 1981; Wang 1982; Willis 1982), most studies are limited in value because of less than optimal blood collection methods and/or inadequate information concerning the length of time animals had been hibernating. In addition, the measurement of steady-state plasma glucose concentrations alone in prior studies did not identify the manner in which the circulating glucose levels are regulated.

Recently, pancreatic A and B cell function has been investigated in euthermic and hibernating hedgehogs (*Erinaceus europaeus*) (Hoo-Paris et al. 1978, 1982, 1983) and dormice (*Glis glis*) (Castex et al. 1979, 1984). Those studies suggest that these hibernating mammals are not capable of responding to intra-arterial injection of glucose. This suggests that plasma glucose concentrations during hibernation may not be regulated by insulin or glucagon secreted from the pancreas. However, as body temperature rises during arousal, pancreatic A and B cell function appears to resume presumably to help regulate plasma glucose concentration (Hoo-
Paris et al. 1983; Castex et al. 1984). The observation that blood glucose is not regulated during hibernation has also been reported for hibernating ground squirrels (Spermophilus undulatus); however, hamsters appear to regulate blood glucose levels throughout hibernation based on plasma glucose and liver glycogen measurements (Musacchia and Deavers 1981). To date, there are no reports of insulin or glucagon manipulated plasma glucose concentrations during hibernation in sciurids.

In the present study, we have investigated pancreatic A and B cell functions in eutherian and hibernating marmots by measuring their respective humoral secretory response to intra-arterial administration of either glucose or arginine. This is the first study that provides information concerning pancreatic regulation of circulating glucose concentrations in a hibernating sciurid.

Material and methods

Animals, surgery and sampling. Five yellow-bellied marmots were trapped in the Elk Mountains of Colorado during the summer 1983, and shipped to Bordeaux, France in early September. (The European marmot is fully protected by law in France; the American marmots were quarantined for 20 days upon arrival). The marmots, weighing 3.0 to 5.0 kg were caged individually, provided with food (sunflower seeds, vegetables), and water ad libitum. Animals were maintained at room temperature (15-20 °C) under natural light throughout October and then moved to a coldroom (5 ± 2 °C) with a photoperiod of 8L:16D, until mid-April.

In early October, an aortic catheter and a thermocouple reentrant tube were permanently implanted in the animals as previously described (Florant and Weitzman 1980). The animals were allowed to recover for 2 weeks at room temperature. After recovery, all animals ceased to feed and food was removed from their cage. Catheters were cleared once per week with heparinized sterile saline (15 units/ml). The catheters of 2 animals were trapped in the Elk Mountains of Colorado during the late summer 1983, and shipped to Bordeaux, France in early September. (The European marmot is fully protected by law in France; the American marmots were quarantined for 20 days after arrival). The marmots, weighing 3.0 to 5.0 kg were caged individually, provided with food (sunflower seeds, vegetables), and water ad libitum. Animals were maintained at room temperature (15-20 °C) under natural light throughout October and then moved to a coldroom (5 ± 2 °C) with a photoperiod of 8L:16D, until mid-April.

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At the beginning of all experiments, a fasted marmot was weighed, and a blood sampling assembly (Florant and Weitzman 1980) was attached to its catheter at least 1 h prior to the study, and a thermocouple was inserted into the reentrant tube. In all of the studies described below, a 1 ml control (C) blood sample was collected at least 1 h prior to injection of glucose or arginine. Subsequent plasma samples from eutherian animals were collected at 0, 5, 10, 20, 30, 60, 90 and 120 min following injection of glucose or arginine and maintained frozen at −20 °C in a vial containing 400 μU of dried Transylol, (CHOAY Laboratory). All plasma samples were analysed for glucose, insulin, and glucagon within one month of the experiment. After each experiment, the red-blood cells were re-suspended in sterile saline and infused back into the animal. The hematocrit in each animal did not vary more than 10% during the experiment.

Euthermic 24 h studies. Before being put into the coldroom, blood samples were collected from five euthermic, fasted marmots, every 3 h over a 24 h period.

Euthermic injection studies. These experiments were performed between bouts of hibernation from November through February. Body temperature (Tb) was continuously recorded to confirm that marmots remained euthermic (Tb ≥ 35 °C) throughout the experiment because ambient temperature (Ta) was 5 °C. After collecting two control blood samples, either glucose (500 mg/kg) or t-arginine (50 mg/kg) in saline was injected intra-arterially into euthermic, fasting marmots (N = 4). Blood samples were collected at time intervals described above, frozen, and later analyzed for glucose, insulin, and glucagon.

Hibernation studies. When an animal entered hibernation or aroused from hibernation, blood samples were collected at the following body temperatures: 35, 30, 25, 15, and 10 °C. When body temperature was below 10 °C (deep hibernation), ten blood samples were collected, with no more than 3 blood samples collected on any given day.

Hibernation injection studies. During a single bout of deep hibernation, animals were injected with either glucose (500 mg/kg) or t-arginine (50 mg/kg). In order to compare the results of hibernation injection studies with euthermic injection studies, post-injection blood sample collection times in hibernating animals were determined on the basis of heart rate (Lyman and Chatfield 1955; Florant and Heller 1977). Normal heart rates for euthermic and hibernating marmots are 120 beats/min and 8 beats/min, respectively. These two heart rates differ by a factor of 15. Therefore deep hibernation blood sample times were calculated on the assumption that total circulation time decreased by approximately this factor; deep hibernation blood samples were collected at 1.25, 2.5, 5, 7.5, 15, 22.5, 30 h, and a later sample collected 3 days after injection.

Analytical methods. Plasma glucose concentration was determined by the glucose oxidase method (Hoo-Paris et al. 1978). Plasma insulin and glucagon levels were determined by radioimmunoassay as previously described (Hoo-Paris et al. 1982). The lower limit of sensitivity was 5 μU/ml for insulin and 10 pg/ml for glucagon. The interassay variation was < 15%. ANOVA and Students t-test were used to analyse the results. The results were considered statistically significant if P < 0.05.

Results

There were no significant changes in plasma glucose, insulin, or glucagon concentration in euthermic, fasted marmots over a 24 h period (Table 1). Plasma glucose concentrations over a 24 h period ranged between 129 ± 10 and 108 ± 7 mg%, with a mean concentration of 118 ± 8 mg%. Plasma insulin values ranged between 25 ± 2 and 16 ± 3 μU/ml, with a mean level of 21 ± 3 μU/ml. Circulating glucagon concentrations fluctuated between 105 ± 11 and 86 ± 13 pg/ml with a mean concentration of 94 ± 6 pg/ml. There was no obvious circadian rhythm of plasma insulin or glucagon in the marmots.

When glucose was injected intra-arterially into euthermic fasting marmots, there was a rapid rise...