Effect of epinephrine upon irreversible disposal and recycling of glucose in dogs[1]

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Summary. Irreversible utilization and recycling of glucose during epinephrine-induced hyperglycemia were studied in adrenal-demedullated dogs exposed to neutral or cold ambient temperature. Whatever the ambient temperature, most of the extra glucose mobilized by epinephrine is recycled rather than irreversibly utilized by the peripheral tissues.

In recent years, many experiments have been devoted to the study of the effects of catecholamines upon glucose production and utilization[3-7]. However, the concept of glucose utilization is ambiguous, because of the extensive recycling of glucose carbon through tricarbon compounds which are taken up by the gluconeogenic tissues and reincorporated into glucose. This amount of promptly recycled glucose is designated as the 'reversible loss'. This possibility of glucose being recycled led us to analyze the metabolic fate of glucose produced in excess under the influence of catecholamines. Is this glucose mostly irreversibly utilized by peripheral tissues through oxidative and non-oxidative pathways, or is it mostly recycled?

Materials and methods. Animals. The experiments were conducted in adrenal-demedullated dogs (ADMX), which were studied under 2 experimental conditions: resting at neutral ambient temperature, with a metabolic rate close to the basal level; exposed to a cold ambient temperature severe enough to induce a 4fold increase in the metabolic rate and a 1.6fold increase in glucose turnover. 17 unanesthetized ADMX female mongrel dogs (8.0–16.2 kg; mean: 11.3 kg), were used in 57 experiments. They were fed daily, with about 300 g of a dry commercial pet food (U.A.R. 121, containing 42.5% of its calories in carbohydrate form). At the start of tracer infusion, the mean fasting time was 20 h, during which tap water was given ad libitum. For 3–5 weeks before the experiments, the animals were trained to lie calmly for at least 345 min on a table set within a large thermostatic chamber, their heads enclosed in ventilated plastic masks[5,8]. 2 ambient temperatures, Ta = + 25 °C and Ta = −21 °C, were selected. At least 4 weeks before beginning the experiments, bilateral adrenomedullary inactivation was accomplished according to Cannon et al.[9]. The dogs remained without any sign of cortical deficiency, for the duration of the experiments, i.e., over a period of 6–12 months. A post-mortem histological examination never disclosed any remaining medullary tissue. About 1 week before the beginning of the experiments, 2 vascular catheters (one in a carotid artery and one in a jugular vein) were chronically implanted[10].

Rectal temperature (Tr) was measured at the beginning and at the end of each experiment. After body mass was noted, the animal was positioned in the experimental set-

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Table 1. Effect of epinephrine infusion (0.1 μg · kg^{-1} · min^{-1}) upon plasma glucose kinetics in dogs exposed to a neutral or a cold ambient temperature

	Ta = + 25 °C (n = 16)			Ta = − 21 °C (n = 13)		
	Before epinephrine	During epinephrine	After epinephrine	Before epinephrine	During epinephrine	After epinephrine
R_{at} Total disappearance rate (μmole · kg$^{-0.75}$ · min^{-1})	50.6 ± 3.21 *	54.9 ± 3.37	52.5 ± 3.19	75.6 ± 3.82	74.7 ± 3.81 *	81,4 ± 3.58
R_{dt} Irreversible disappearance rate (μmole · kg$^{-0.75}$ · min^{-1})	37.5 ± 2.72	36.8 ± 2.25	35.2 ± 2.18	61.8 ± 3.98 **	55.0 ± 3.20 **	63.2 ± 3.36
$R_{at} - R_{dt} = R$ Glucose recycling (μmole · kg$^{-0.75}$ · min^{-1})	13.1 ± 1.04 ***	18.2 ± 1.47	17.3 ± 1.47	13.8 ± 1.19 ***	19.6 ± 1.56 *	18.3 ± 1.21
$\frac{R}{R_{at}}$	0.26 ± 0.018 ***	0.33 ± 0.016	0.33 ± 0.015	0.19 ± 0.018 ***	0.26 ± 0.019 **	0.23 ± 0.016
Plasma glucose (mg · 100 ml^{-1})	87.4 ± 2.19 ***	99.8 ± 3.07	98.2 ± 2.67	89.9 ± 3.40 ***	119.4 ± 3.91 ***	100.1 ± 2.44

Values are means ± SEM which are significantly different at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (paired t-tests). n = Number of experiments.

up. An initial adjustment period of 45 min was allowed before beginning infusion, in order to obtain metabolic steady state and thermal equilibrium. During the experiments, the respiratory mask was connected to an open-circuit system[11] for measurement of 0_2 consumption (Paramagnetic analyzer, Magnos 2, Hartmann-Braun).

Tracer methodology. Reversible loss of glucose can be calculated by the difference between the disappearance rates measured with 2 tracers[12-14]. The tracer used for calculation of total glucose disappearance must have its label completely lost at an early stage of metabolism. The tritium of D-(2-^{3}H) glucose being lost as water at the hexose-6-phosphate stage, this tracer is suitable for such a determination. On the other hand, each molecule deriving from U-^{14}C glucose remains labelled throughout the different pathways of recycling. As a consequence, this second tracer is suitable for the measurement of irreversible loss.

Experiments were carried out according to the priming dose-infusion technique[15]. U-^{14}C glucose and D-(2-^{3}H) glucose diluted in saline were simultaneously employed. The priming dose was followed by a continuous infusion of the same solutions at a rate of 0.1 ml/min^{-1}. This infusion began at t = 0 and continued until the end of the experiment, i.e., for 300 min. The total time of the experiment was 345 min (including the initial adjustment period). The priming dose/infusion rate ratios were 120:1 and 90:1 at neutral and cold ambient temperatures, respectively. Simultaneously, a saline infusion (0.1 ml · min^{-1}) was administered through the same jugular vein, between t = 0 and t = 167 min (pre-epinephrine control period) and between t = 242 and t = 300 min (post-epinephrine control period). Between t = 167 and t = 242 min, solution of L-epinephrine chlorhydrate (Adrenaline Rousse-Uclaf) was substituted for saline and infused (0.1 ml/min^{-1}) in doses of 0.1 and 0.5 $\mu g \cdot kg^{-1}$ min^{-1}.

Arterial blood samples were drawn at t = 75, 105, 135, 165, 180, 195, 210, 225, 240, 255, 270 and 295 min in chilled tubes containing a NaF-heparine mixture. Plasma glucose concentration was measured by a glucose oxidase method. Plasma glucose ^{14}C and ^{3}H S.A. were calculated by the method of Issekutz et al.[16], the 2 radioisotopes being separated by the method of Hetenyi and Reynolds[17]. For each of the 2 tracers, the rate of glucose utilization (rate of disappearance, Rd) was calculated according to Steele's equations[18,19]. The volume distribution of glucose was assumed to be 300 ml · kg^{-1} body mass, and the pool size was calculated from the plasma glucose concentration. The rate of glucose recycling was obtained as the difference between the total disappearance rate (Rdt), as measured by (2-^{3}H) glucose, and the irreversible loss (Rdi), as measured by U-^{14}C glucose.

The mean values of Rdt and Rdi, calculated for the time intervals of 75–105 min, 105–135 min and 135–165 min, were considered as 'before epinephrine'. In order to avoid errors due to metabolic and physiological changes caused by the onset of epinephrine infusion, Rdt and Rdi were not used to calculate mean values during the time interval between t = 165 and t = 180 min. Therefore, the mean values of Rdt and Rdi calculated for the time intervals of 180–195 min, 195–210 min, 210–225 min and 225–240 min, were considered as 'during epinephrine'. Similarly, the mean values of Rdt and Rdi calculated for the time intervals of 255–270 min and 270–295 min were considered as 'after epinephrine'. Statistical analysis was performed by using the matched data method.

Results. The dogs remained normothermic throughout these experiments, the mean (± SEM) Tr at the end of the experimental period being 38.8 ± 0.08 °C and 38.0 ± 0.13 °C at Ta = + 25 °C and Ta = −21 °C, respectively. Mean 0_2 consumption (VO_2), measured between t = 90 and t = 150 min (before epinephrine infusion), was 14.0 ± 0.30 ml · kg$^{-0.75}$ · min^{-1} at Ta = + 25 °C, this value being close to the BMR observed in this species. At Ta =

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Table 2. Effect of epinephrine infusion (0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) upon plasma glucose kinetics in dogs exposed to a neutral or a cold ambient temperature

	Ta = + 25 °C (n = 14)			Ta = − 21 °C (n = 14)		
	Before epinephrine	During epinephrine	After epinephrine	Before epinephrine	During epinephrine	After epinephrine
R_{dt} Total disappearance rate ($\mu mole \cdot kg^{-0.75} \cdot min^{-1}$)	48.2 ± 3.06 ***	72.3 ± 4.76	70.6 ± 3.16	77.2 ± 4.65 ***	108.2 ± 6.19	104.2 ± 4.80
R_{di} Irreversible disappearance rate ($\mu mole \cdot kg^{-0.75} \cdot min^{-1}$)	33.9 ± 2.04 **	41.5 ± 2.06	45.4 ± 2.45	61.5 ± 4.24 *	72.4 ± 4.11 *	81.7 ± 4.23
$R_{dt} - R_{di} = R$ Glucose recycling ($\mu mole \cdot kg^{-0.75} \cdot min^{-1}$)	14.3 ± 1.90 ***	30.8 ± 3.82 **	25.2 ± 2.89	15.7 ± 1.14 ***	35.8 ± 2.96 ***	22.5 ± 1.73
$\frac{R}{R_{dt}}$	0.29 ± 0.029 ***	0.41 ± 0.030 **	0.35 ± 0.034	0.21 ± 0.016 ***	0.33 ± 0.020 ***	0.22 ± 0.014
Plasma glucose ($mg \cdot 100\ ml^{-1}$)	82.8 ± 3.49 ***	133.5 ± 5.74 ***	107.4 ± 4.66	80.9 ± 3.74 ***	164.2 ± 11.83 ***	95.7 ± 4.17

Values are means ± SEM which are significantly different at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (paired t-tests). n = Number of experiments.

–21 °C, mean VO_2 was 4.2 times greater and reached 58.7 ± 1.61 $ml \cdot kg^{-0.75} \cdot min^{-1}$.

With the smaller dose of epinephrine (0.1 $\mu g \cdot kg^{-1} \cdot min^{-1}$), (table 1), in dogs resting at neutral ambient temperature, there was just a slight (+8%), but significant ($p < 0.05$), increase in the glucose total disappearance rate (Rdt). This increase was not due to an increase in irreversible loss (Rdi), which remained unchanged, but it can be entirely accounted for by an increase in glucose recycling (+ 39%, $p < 0.001$). In the cold ambient temperature, Rdt remained unchanged, because of a balance between Rdi, which was significantly (–11%, $p < 0.01$) decreased, and recycling, which was significantly (+ 42%, $p < 0.001$) increased. Therefore, this smaller dose of epinephrine always increased recycling, but specially in cold it decreased the irreversible utilization.

With the larger dose of epinephrine (0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$), (table 2), in dogs resting at neutral ambient temperature, there was a large increase (+ 50%, $p < 0.001$) in Rdt. This increase was mainly due to an increase in recycling (+ 115%, $p < 0.001$) which accounted for about $^2/_3$ of the increase in Rdt. Irreversible loss was less, but significantly (+ 22%, $p < 0.01$), increased by the hormonal infusion. In dogs exposed to cold, a similar situation was observed, with a significant increase (+ 40%, $p < 0.001$) in Rdt, which was mainly ($^2/_3$) due to a large (+ 128%, $p < 0.001$) increase in recycling. Therefore, this larger dose of epinephrine induced an increase in irreversible glucose loss, but this increase was smaller than that observed for recycling.

Discussion. The catecholamine secretory response to cold exposure is greatly decreased in ADMX dogs [20,21], while basal concentrations of plasma cortisol, as well as the adrenocortical response to cold stress, were not impaired. The doses of epinephrine used in the above experiment were chosen on the basis of data collected by Klepping et al.[22], who observed, by catheterization of the adrenal veins in dogs, that the output of catecholamines increased to 0.3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ during cold exposure.

Irreversible loss of glucose includes transformations such as oxidation to CO_2, along with conversions into metabolites which are incorporated in molecules with relatively long turnover times with regard to the duration of the experimental period. Unfortunately, in the above experiment, precise measurement of the oxidation rate is hindered by the decrease in the bicarbonate pool, which, in turn, is due to the epinephrine-induced hyperlactatemia. On the other hand, reversible loss consists of transformations which occur through utile and futile cycles[23]. The above experiment, conducted in dogs, at an energy expenditure level close to the BMR, shows that most of the extra glucose mobilized by epinephrine is recycled, rather than actually utilized. More surprinsingly, a similar situation occurred in cold-exposed dogs, even though carbohydrate needs for thermoregulation increase in cold ambient temperature[24]. These needs are probably partially covered by intramuscular glycogenolysis which is also enhanced by epinephrine.

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Effect of antiblastokinin on rabbits pregnancy*

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Summary. On different days following coitus, i.e., during the first stages of pregnancy, adult female rabbits were treated with antiblastokinin obtained from a chicken. A high prenatal mortality was observed and a reduction of the weight in the stillborn of the rabbits treated. The antiblastokinin produces evident biological effect about the 4th day following coitus, during the period of maximum production of blastokinin by the endometrium in vivo.

Blastokinin (BKN) is a protein isolated by the uterine secretion of various mammals during the estrus or the early days of pregnancy or pseudopregnancy[1–6]. In the rabbit it is present between days 3–9 following copulation, reaching maximum about the 5th day and it appears to favour the development and implantation of fertilized ovum[3–9]. Krishnan[10] and Daniel[11] have demonstrated in the rabbit and the pig that the administration of antiblastokinin (anti-BKN) in the first stages of embryonic development, in particular during the period of preimplantation of the blastocysts, interferes with the number of foetus born[10,11], duration of gestation and appearance of successive estrus[11]. The aim of our research is to establish the biological effect of anti-BKN found by us in the chicken[12] and to establish at what time the course of administration of anti-BKN determines evident biological effect.

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