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Hormonal Stimulation of Substrate Utilization in Brown Adipose Tissue of Cold Acclimated Rats (38443)

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(Introduced by J. Le Blanc)

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The occurrence of nonshivering thermogenesis (NST) in cold acclimated rats is widely recognized. Its magnitude seems to depend, in part, on the presence of brown adipose tissue (BAT) (1), but the mechanisms of the thermogenesis are not well known. Only a few experiments on substrate utilization by BAT have been performed *in vivo* (2, 3). Cold acclimated rats become hypersensitive to the calorigenic effect of norepinephrine (NE) when tested at neutral temperature (4, 5). The enhanced calorigenesis probably depended upon an enhanced utilization of mobilized fatty acids. The lipolytic effect of NE in BAT is mediated by cyclic-AMP (6, 7), but it seems that cold adaptation leads to a reduced activation of cAMP system by NE (8, 9). Intermittent exposures to cold (10), or acclimation to fluctuating temperatures (5) also increase sensitivity to NE, but somewhat less than continuous exposure.

The aim of this investigation was to study, *in vivo,* substrate uptake or release in the interscapular BAT of the cold acclimated adult rat and to measure the effects of NE infusions. As this action could be mediated by cyclic AMP, the effects of infused dibutyryl-cyclic AMP (DBcAMP) were also studied and compared to those obtained with NE.

Methods. The method we used was adapted from that described by Hardman and Hull (3), on the newborn rabbit. All the experiments were performed on 5 mo old male Long Evans rats. The control group was maintained at ambient temperature of 28°, one experimental group was adapted to a constant cold temperature (5°) (CA group) and another group was acclimated to a fluctuating temperature according to a programmed nycthemeral cycle from 5 to 28° (Cy group). In the last condition, the temperature was stable at 5° from midnight to 6 AM and at 28° from noon to 6 PM. It took 6 hr to change from one temperature to the other. All the rats were subjected to 12 hr of artificial lighting from 6 AM to 6 PM. The animals were maintained in these thermal conditions for 3 or 4 mo. Prior to the test, the rats were fasted for 7 hr, anesthetized with nembutal (40 mg/kg/ip) and kept at their neutral temperature. Three catheters were introduced into one carotid, the Sulzer's vein and one jugular vein. Fifteen minute infusions of NE (8 μg/kg/min) or of DBcAMP (0.3 mg/kg/min) were performed through the jugular vein. This infused dose of NE had been shown to increase the oxygen consumption by about 100% in CA, 60% in Cy and 30% in control rats. The infused volume was 2.25 ml. To take into account the plasma dilution due to the infused volume, the initial values were measured on the same animals at the end of a 2.25 ml saline infusion. Venous and arterial blood samples [from carotid (0.5 ml) and Sulzer's vein (0.5 ml)], were collected at the end of the saline and drug infusions and 45 min after the end of the infusion. So, during the experiment, the difference between total infused and collected volumes was small (1.5 ml). NE and DBcAMP infusions were performed on different groups of rats (about 20 animals in each group).

Arteriovenous differences (A−V) in glucose, free fatty acids (FFA) and glycerol plasma contents were calculated. The blood flow through the venous catheter was measured by determination of the time necessary to collect 0.5 ml of blood. Because the weight of BAT was not the same, according to the acclimation conditions, we expressed the substrate uptake or release per gram of wet weight of BAT (A − V difference × blood flow/BAT w/w). This expression allows us to compare the metabolism per unit mass of tissue. The plasma glucose level was measured by the *o*-toluidine method, the FFA by a method modified from Novak (11) and the glycerol using a microenzymatic method (12). In all the quantitative comparisons, a P value < 0.05 was accepted as significant.

Results. The weight of interscapular BAT (276 ± 25 mg in controls) was twofold higher in the Cy group (540 ± 38 mg) and 2.5 in the CA group (745 ± 40 mg).

Before the infusions, in both experiments, the mean blood flow through the catheter was the same in control and Cy groups (about 150 μl/min), but it was significantly greater in the CA group (200 μl/min) (Fig. 1). The mean glucose arterial levels were not different in the three groups (1.20–1.25 mg/ml) (Figs. 2 and 3). The plasma glucose uptake into BAT was, also, quite similar (20–40 μg/g/min). Glycerol was released from BAT in controls (50 n*M*/g/min) and in Cy rats (20 nmoles/g/min) but not in CA rats. The level of FFA in arterial blood was about 1.2 μmoles/ml in all groups. In controls, FFA were released from BAT (50 nmoles/g/min); inversely, there was an uptake of blood FFA in Cy (40 nmoles/g/min) and CA (70 nmoles/g/min) groups (Figs. 2 and 3).

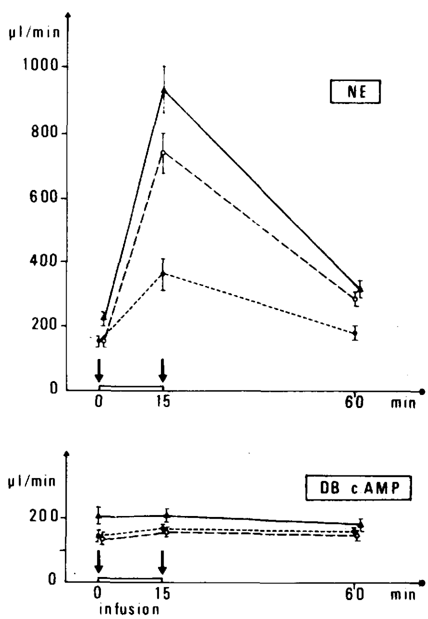

FIG. 1. Effects of norepinephrine or DBcAMP infusions on the blood flow through the catheterized Sulzer's vein. The infusion duration is indicated between arrows. ●.......● control group, ○-----○ Cy group, ▲———▲ CA group. Standard deviations are shown.

Following the NE infusion, the blood flow through BAT was enhanced (2.4 times in controls and more than four times in CA and Cy rats) (Fig. 1). At the end of the infusion, the arterial level of glucose was doubled but it has returned to normal 45 min later. The uptake of glucose into BAT was enhanced similarly in the three groups (65–95 μg/g/min) (Fig. 2). The glycerol plasma level was raised by about 120% in control and CA rats and by 180% in Cy group. The significant increase of glycerol release from BAT had the same magnitude in all the groups (170–240 nmoles/g/min). Except in controls, this effect was significantly abolished 45 min after the end of the infusion (Fig. 2). In the arterial blood, the FFA plasma levels were significantly increased by about 60% in control and Cy rats and nonsignificantly (20%) in CA rats. The FFA coming from triglycerides (TG) hydrolysis in BAT were, in part, released into the blood: this release was greater in control rats (1600 nmoles/g/min) than in cold acclimated ones (180 (CA) and (Cy), 250 nmoles/g/min) (Fig. 2).

In all the groups, the blood flow was not enhanced at the end of the infusion of DBcAMP (Fig. 1). There was a twofold increase in the arterial levels of glucose (Fig. 3) and this effect had disappeared after 45 min. Inversely, the significant decreases of blood glycerol (25–40%) and FFA (about 50%) were of longer duration (Fig. 3). In BAT, the uptake of glucose which was slightly but significantly positive before the infusion (about 30 μg/g/min), was significantly enhanced in the three groups. This increase is twice larger in controls and Cy group (150 μg/g/min) than in CA (70 μg/g/min) (Fig. 3). The DBcAMP infusion had no significant effect on the release of glycerol. In control rats, the release of FFA was inhibited. In the other two groups, the uptake of circulating FFA by BAT was completely abolished; this phenomenon was still observed 45 min after the end of the infusion (Fig. 3).

Discussion. The catheterization of Sulzer's vein a few days before the experiments was not possible. The measures were, hence, performed in anesthetized animals kept at thermal neutrali-

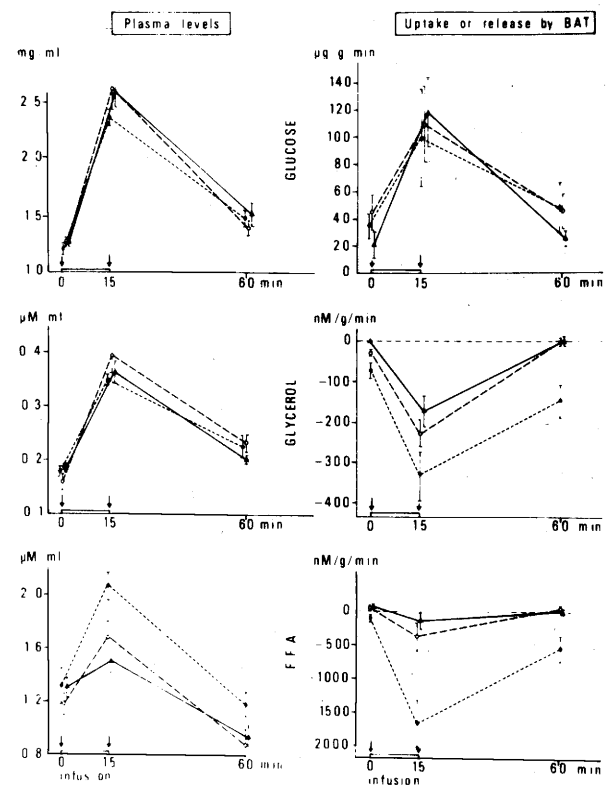

FIG. 2. Effects of 15 min iv infusions of norepinephrine (8 μg/kg/min) on the metabolism of glucose, glycerol and FFA. On the left part of the figure, the variations of arterial substrate levels are plotted. On the right part, the uptake (> 0) or the release (< 0) of the substrates in interscapular BAT are plotted. ●.......● control group, O----O Cy group, ▲———▲ CA group. Standard deviations are shown.

ty. In that thermic condition, the energetic responses to NE infusions are maximal. Sulzer's vein being the main vein of interscapular BAT but not the only one, the total amount of substrates retained or released from the tissue could not be measured. Then, between the three acclimation groups, only a comparison of the quantitative effects of NE or DBcAMP can be made.

The measured blood flow was about 30% greater in CA rats than in controls. This fact is certainly related to the hypertrophy and the hypervascularization of the tissue. At the end of NE infusions, the larger enhancement of the blood flow in CA rats, first observed by Evonuk and Hannon (13), is likely due to the hypersensitivity to NE (14). We found a similar hypersensitivity in Cy rats. The DBcAMP infusions did not affect the blood flow through this tissue; this fact can be related to the lack of general calorigenic effect of the drug we previously observed.

DBcAMP of NE infusions cause a twofold increase in the circulation level of glucose, probably by stimulating liver phosphorylase kinase. NE significantly stimulates the uptake of glucose by BAT, in all the groups. The magnitude of enhancement is almost proportional to the increase of glucose plasma level but not to the increase of general energetic metabolism which is three times more increased in CA rats and twice in Cy rats than in controls. Except in CA rats, DBcAMP has a greater effect than NE on the uptake of glucose. This fact can be due to a release of insulin resulting from cAMP stimulation of pancreatic cells or following hyperglycemia (15).

The study of glycerol release allows estimation of the degree of TG hydrolysis in the white adipose tissue in which the glycerokinase activity is very low. However, in BAT, a more important enzyme activity possibly exists but was not found by all the investigators (16). Exogenous

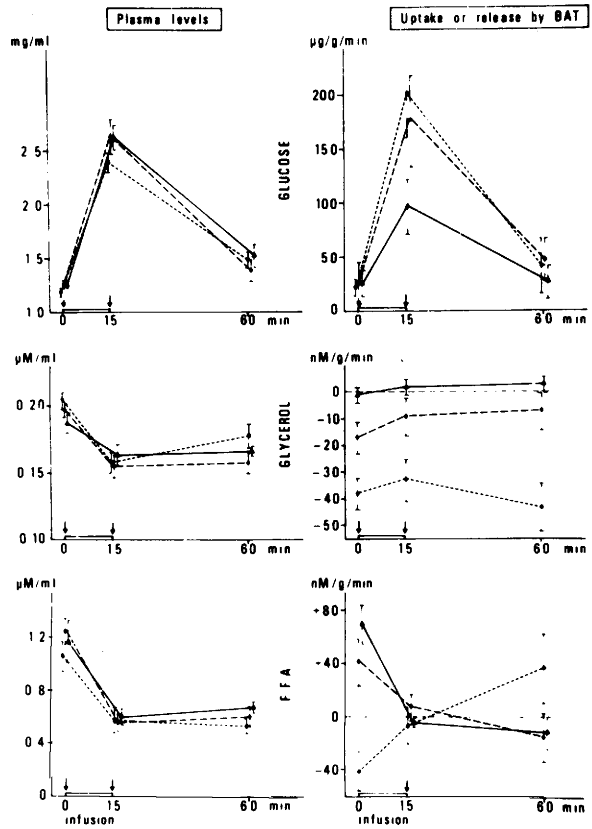

FIG. 3. Effects of 15 min iv infusions of DBcAMP (0.3 mg/kg/min) on the substrate metabolism. Other conditions are the same as in Fig. 2.

NE produced a very important general lipolysis as is shown by the increase of plasma glycerol levels. On the contrary, DBcAMP induced a decrease in the intensity of general lipolysis in the three groups as it is shown by the decrease of plasma glycerol levels. This antilipolytic effect has been reported (18) and was attributed to a stimulation of insulin release. In the BAT of control and Cy rats, there was, before infusions, a small lipolysis certainly due to their 7 hr fasting. No lipolysis was noted in the BAT of the CA animals. In the Cy and CA groups, an uptake of plasma FFA was found. It can be concluded that, although the rats were kept at thermal neutrality, the energetic metabolism per gram of BAT is enhanced in the two groups of cold acclimated rats.

Following intravenous infusions of DBcAMP, the uptake of plasma FFA by BAT of CA and Cy rats has disappeared, while the magnitude of lipolysis in the tissue, shown by glycerol release from BAT, was not affected. In fact, the infusion of DBcAMP did not decrease lipolysis in BAT as it did in other stored fats. If this latter effect is mediated by released insulin, it is possible that this hormone does not act in BAT as it does in white fat. Indeed, insulin infusions to newborn rabbits (18) did not affect lipolysis in BAT. The effect of DBcAMP infusions cannot be easily explained. *In vitro,* this derivative penetrates the cell membranes and activates the kinases (17). But, when administered *in vivo,* its numerous general effects (hormone synthesis, glucose mobilization, etc.) can counteract its local action.

Following the NE infusions, it appeared a lesser release of FFA from BAT of the two groups of cold acclimated rats. This fact can be due either to a smaller lipolysis in the tissue or to a greater utilization by BAT. The release of glycerol indicates that the enhancement of lipolysis intensity (per gram of BAT) has almost the same magnitude in the three groups; then, there is a greater FFA utilization in cold acclimated rats. If we postulate that hydrolyzed glycerol is not reutilized, the molar ratio of glycerol and FFA release (FFA/glycerol) allows calculation of the utilization of FFA mobilized in BAT by NE. In control rats, this ratio was about five, indicating a lack of oxidation of the hydrolyzed FFA. The fact that the ratio is greater than three could be explained by incomplete TG hydrolysis or partial glycerol reutilization. Thus, it would seem that, in response to NE, the BAT acts as a lipid store somewhat like the white adipose tissue; we have seen that the lipid contents of the two tissues are not very different (unpublished results). On the contrary, in the cold acclimated groups, the ratio is close to 1 in CA and about 1.5 in Cy rats. In these two groups, a large part of the FFA hydrolyzed by NE in BAT, seems to be utilized in the tissue. If it is supposed that glucose and FFA retained by the tissue are utilized for energetic expenditure, the respective part of the two substrates can be roughly estimated. In controls, the calorigenic expenditure due to NE infusion is only supplied by glucose utilization (the hydrolyzed FFA were released). In the CA group, glycerol and FFA releases indicate that TG hydrolysis liberates $180 \times 3 = 540$ nmoles/min of FFA. Only 200 nmoles/min are released into the blood. The difference (340 nmoles/min = 88 μg/min) is utilized in the tissue. In the same time, we found a utilization of 95 μg/min of glucose. As the calorigenic potency of FFA is about twice that of glucose, the part of NE enhanced expenditure which is supplied by FFA is twice that supplied by glucose. By the same calculation, we find, in the Cy group, a similar energetic supply of glucose and FFA. In these calculations, we postulate that the hydrolyzed glycerol is not reutilized. But, if a part of glycerol is reutilized, there is a greater TG hydrolysis in BAT and an amount of FFA greater than the calculated amount is really utilized in BAT of the cold acclimated rats. In both cases, as the glucose uptake enhancement per gram of BAT was similar in the three groups, it can be assumed that, in BAT of rats acclimated to constant or intermittent cold, the part of NE calorigenic effect related to acclimation (total effect − effect in controls) is supplied by FFA mobilized from the tissue.

Summary. A study of glucose, glycerol and free fatty acid (FFA) uptake and release in the interscapular brown adipose tissue (BAT) was done on rats acclimated to constant cold (CA), fluctuating cold (Cy) or a control ambient temperature. At thermal neutrality the arteriovenous differences and the blood flow through the tissue were estimated. The effects of norepinephrine (NE) or dibutyryl cyclic-AMP (DBcAMP) were measured. Before infusions an uptake of plasma FFA by BAT was found in CA and Cy rats, and there was a release in the controls. Plasma glucose was taken up by the BAT of the three groups of rats. DBcAMP infusions did not enhance blood flow and lipid metabolism in BAT but increased glucose uptake in all the groups. By the end of a NE infusion, blood flow was increased 2.4-fold in controls and four- to fivefold in CA and Cy rats. Glucose uptake and glycerol release were greatly increased in all the groups. In controls hydrolyzed fatty acids were released in the venous blood, but in CA and Cy rats most of them were retained in the tissue. Therefore, in the controls BAT seems to be essentially a reserve of lipids which can be mobilized by NE. In the two groups of cold acclimated animals, the fatty acids hydrolyzed by NE, which are retained in the tissue, may be the substrate of the NE dependent increase of tissue energetic metabolism.

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