Influence of Dibutyryl Cyclic 3′,5′-AMP on Glucose and Fat Metabolism in Normal and Diabetic Rats

Cyclic 3′, 5′-AMP (cAMP) is involved in a wide variety of apparently divergent processes ranging from activation of enzymes, such as phosphorylase, to increasing adipose tissue lipolysis, steroid hydroxylation and the activation of the secretory mechanisms of some peptide hormones, particularly of insulin[1].

Since there are only a few reports about the metabolic actions of cAMP and its derivatives in intact rats, our purpose was to investigate the lipolytic, glycogenolytic and insulin stimulating activity of a cAMP-analog, the 6-N, 2′-*O*-dibutyryl-derivative (DBA), which has shown a greater effectiveness both in vivo and in vitro, probably due to a greater lipid solubility or resistance to inactivation by phosphodiesterase[1].

Three groups of 5 male Sprague-Dawley, non-fasting rats, weighing g 250 ± 30, were injected with DBA i.p. at the doses of 10–50 and 100 mg/kg, 30 min before killing. Blood samples were taken for analysis of blood glucose by a chemical method (*o*-toluidine reaction)[2], of plasma free fatty acids by Dole method modified by Trout et al.[3], of glycerol by the enzymatic assay of Wieland[4] and of plasma immunoreactive insulin by the double antibody method of Hales and Randle[5]. In a second series of experiments, we performed the same schedule in streptozotocin diabetic rats: the animals were given a single injection of 65 mg/kg of streptozotocin i.v., 6 days before the experiments; the rats had free access to food and water throughout the week, during which they lost about 30 g in weight.

Our results are reported in Table I: normal rats treated with DBA showed a significant increase ($p < 0.01$) of blood glucose concentrations and plasma IRI levels. On the contrary, plasma glycerol and FFA concentrations were markedly decreased by DBA ($p < 0.01$).

The result of FFA and glycerol depression, i.e. antilipolysis instead of lipolysis observed in vitro[1,6,7], induced us to repeat the same experiment in streptozotocin diabetic rats[8,9], in order to clarify whether hyperinsulinemia was responsible of the aforementioned antilipolytic effect of DBA.

In streptozotocin diabetic rats there was hyperglicemia together with a normal range of plasma IRI levels but, while DBA produced a further increase of blood glucose concentrations, it was not able to modify plasma IRI. Nevertheless plasma glycerol and FFA decreased after DBA treatment, showing at the highest doses the same pattern observed in normal rats (Table II).

Our results show that DBA injected in vivo increases both glucose and insulin levels, probably through separate mechanisms, as already observed in primates[10] and in rats[6]. The FFA and glycerol depression induced by DBA in normal rats is in agreement with similar results obtained by Bieck et al.[6]; but, while these authors observed no increase in blood glycerol concentrations in alloxan diabetic rats following DBA injection, our experiments show a marked fall of glycerol as well as of FFA: this finding might be due to the different diabetogenic drugs used: alloxan diabetes in rats is characterized by a striking elevation in the circulating concentration of

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Table I. Effect of DBA on blood glucose, glycerol, FFA and IRI of normal rats

	Blood glucose (mg/ml)	Blood glycerol (μmole/ml)	Plasma FFA (μEq/ml)	Plasma IRI (μU/ml)
Controls	1.07 ± 0.06	0.094 ± 0.007	0.36 ± 0.03	18.8 ± 0.73
DBA (10 mg/kg)	1.66[a] ± 0.08	0.054[a] ± 0.004	0.19[a] ± 0.015	32[a] ± 1
DBA (50 mg/kg)	1.69[a] ± 0.08	0.036[a] ± 0.003	0.15[a] ± 0.01	35.6[a] ± 1.33
DBA (100 mg/kg)	1.89[a] ± 0.09	0.036[a] ± 0.003	0.19[a] ± 0.016	36[a] ± 0.8

Mean values ± SE. [a] p-values < 0.01 compared with controls. The mathematical analysis was carried out by Students t-test.

Table II. Effect of DBA on blood glucose, glycerol, FFA and IRI of streptozotocin diabetic rats

	Blood glucose (mg/ml)	Blood glycerol (μmole/ml)	Plasma FFA (μEq/ml)	Plasma IRI (μU/ml)
Controls	2.94 ± 0.1	0.071 ± 0.006	0.38 ± 0.03	17.4 ± 0.8
DBA (10 mg/kg)	3.13 ± 0.15	0.093 ± 0.009	0.29 ± 0.03	18 ± 1.09
DBA (50 mg/kg)	3.55[a] ± 0.20	0.035[a] ± 0.002	0.19[a] ± 0.01	23 ± 1.34
DBA (100 mg/kg)	3.46[a] ± 0.2	0.033[a] ± 0.002	0.20[a] ± 0.01	18 ± 1.22

Mean values ± SE. [a] p-values < 0.01 compared with controls. The mathematical analysis was carried out by Students t-test.

glucose, FFA and ketones, while in streptozotocin diabetes there is hyperglycemia but plasma FFA and blood ketones are not significantly elevated[8]. On the other hand, our results are in agreement with those of ALTSZULER et al.[11] in dogs. Therefore it is evident that decreased lipolysis by DBA can occur in the absence of increased insulin secretion.

Up to now, the only antilipolytic hormones known are insulin and prostaglandins: since we must reject the hypothesis of insulin being responsible for the DBA antilipolytic effect observed in vivo, we might consider the hypothesis of a prostaglandin-like substance. Ho et al.[12] observed that a hormone antagonist is formed in fat cells during hormone action; the effect of this antagonist can be mimicked by prostaglandin E_1 and E_2[12]; moreover prostaglandins are released from adipose tissue in response to hormonal stimulation[13].

Since both cAMP and DBA mimicked the action of hormones to promote antagonist formation[12], it seems likely that antilipolysis by DBA in vivo might be due to excessive production of the antagonist itself which might therefore be able to overcome the direct action of the low concentrations of cyclic nucleotides which can reach fat cells in vivo.

Riassunto. La somministrazione di DBA a ratti sia normali che resi diabetici con streptozotocin provoca un persistente effetto antilipolitico, indipendente dai valori di insulina plasmatica.

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Pentobarbital Anaesthesia. Effects on Blood Sugar, Serum Immunoreactive Insulin and Free Fatty Acid Responses to Glucose[1]

These studies were made in dogs, basally and during an intravenous glucose tolerance test.

Material and methods. 7 male and 1 female mongrel dogs, weighing 9–16.3 kg, fed on dog chow pellets and water ad libitum were used. After 17–22 h fast, they were anaesthetized (sodium pentobarbital, 33 mg/ml aqueous solution, 1 ml/kg body wt., rapid i.v. injection). Tests were performed 1 h later; glucose was rapidly injected in femoral vein (1 g/kg body wt., 20% aqueous solution), and blood was withdrawn (femoral vein) at several intervals thereafter. Control experiments using unanaesthetized dogs were carried out (self-control design).

Blood samples were assayed for blood sugar (BS) (Technicon Autoanalyzer[2]), both serum immunoreactive insulin (IRI)[3] and free fatty acids (FFA)[4]. All results were analyzed for variance[5].

Results. As shown in the Figure, BS, serum IRI and FFA basal levels remained unaffected by anaesthesia.

The BS peak during the test in the unanaesthetized dogs was higher than in controls ($p < 0.001$ between 5 and 25 min, $p < 0.01$ at 45 min). Glucose disappearance from blood during the test followed an exponential law in unanaesthetized dogs, as shown by a high, highly significant correlation coefficient for the relationship shown in the Table. Previous reports were thus confirmed[6]. The law followed in anaesthetized dogs was also exponential and similar in slope, but their y means differed (see Table). Therefore, glucose space appeared to be modified by pentobarbital.

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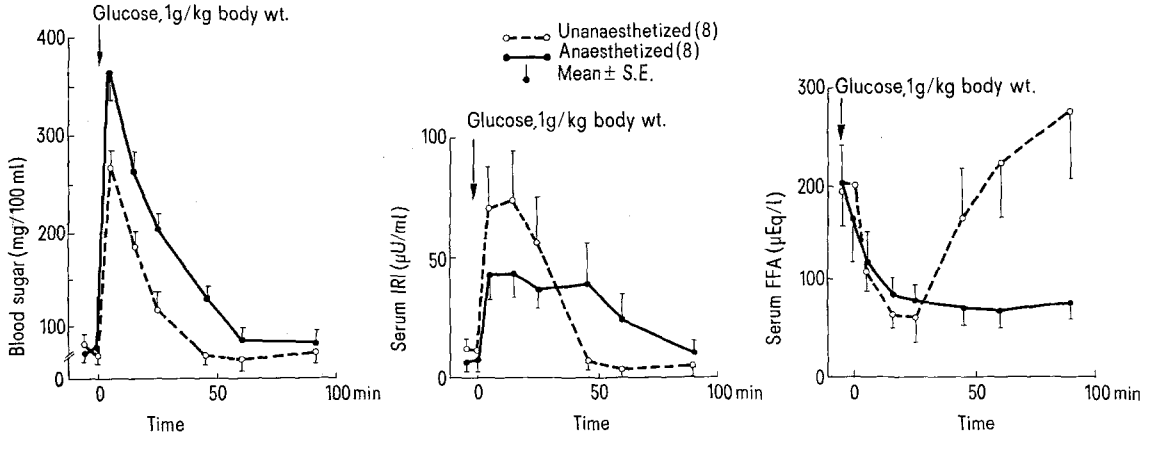

Some effects of pentobarbital anaesthesia. Number of animals per group in parenthesis.