STIMULATION OF ADIPOSE TISSUE TRIGLYCERIDE LIPASE ACTIVITY AFTER IN VIVO ADMINISTRATION OF DIBUTYRYL CYCLIC AMP OR NOREPINEPHRINE

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Received 8 August 1973
Revised version received 28 August 1973

1. Introduction

The key role of cAMP* in mediating hormonal stimulation of lipolysis is generally accepted [cf. 1, 2]. The lipolytic action of cAMP and especially of its dibutyryl derivative is well documented in vitro [2, 3], whereas the in vivo administration of cAMP failed to produce any significant increase in plasma FFA in rabbit or rat [4], significantly decreased plasma FFA in dog [4] and exerted a biphasic effect on plasma FFA when administered to man [5]. In rats, both normal and alloxan diabetic, DBcAMP did not elevate either plasma FFA or glycerol but exerted a significant antilipolytic effect [6]. These results are in apparent contradiction with the lipolytic effects of cyclic nucleotides on adipose tissue lipase activity in vitro. Moreover, it is well known that in vivo administration of catecholamines, the effect of which is mediated by cAMP, regularly elevated not only blood glucose level but also plasma FFA [7]. The situation is further complicated by the fact that cAMP, but not DBcAMP, was found recently to have an antilipolytic effect in isolated adipocytes [8].

In an attempt to clarify the primary effect of cyclic nucleotides on adipose tissue in vivo, it was thought worthwhile to compare the dynamics of adipose tissue triglyceride lipase activity after the administration of DBcAMP (the lipolytic action of which is in vitro not contested) with that of norepinephrine (a relatively pure β-agonist) in the presence and absence of two antilipolytic agents, insulin and nicotinic acid.

* *Abbreviations*: cAMP, cyclic AMP; DBcAMP, dibutyryl cyclic AMP; FFA, free fatty acids.

2. Materials and methods

Male Sprague-Dawley or Wistar rats weighing 200–250 g fed with a standard diet were used. The animals were anesthesized by intraperitoneal injection of 0.8–1.0 ml of Numal (10% diethylaminoallyl isopropyl barbituric acid) per kg body weight. To minimize the endogenous epinephrine production, hexamethonium chloride 40 mg/kg body weight was included in the injection. The animals were kept under anesthesia for 20 min and then two 0.5 cm openings were made in the region of the external inguinal ring to facilitate access to fat pads. After a further 10 min of anesthesia 15–30 mg of the control samples of epididymal adipose tissue were taken. The animals were than injected intraperitoneally with the appropriate drug, DBcAMP or norepinephrine with or without insulin or nicotinic acid, and at various time intervals after the injection further samples of epididymal adipose tissue were taken and immediately frozen on dry ice. The adipose tissue samples were quickly weighed and homogenized in an equivalent volume of ice-cold buffered sucrose solution (0.25 M sucrose in 10 mM Tris–HCl buffer pH 7.4 containing 1 mM EDTA) and frozen immediately by immersion of the test tubes in alcohol cooled with dry ice. The conditions of homogenization and centrifugation of the homogenate were described elsewhere [10]. The fat cake was discarded and the clear 1200 *g* infranant was taken as the enzyme preparation.

In experiments where the effect of cAMP was tested in vitro, the enzyme preparation was preincubated for 10 min at 30°C in the presence of the following complete system: 0.05 M Tris–HCl buffer pH 7.4,

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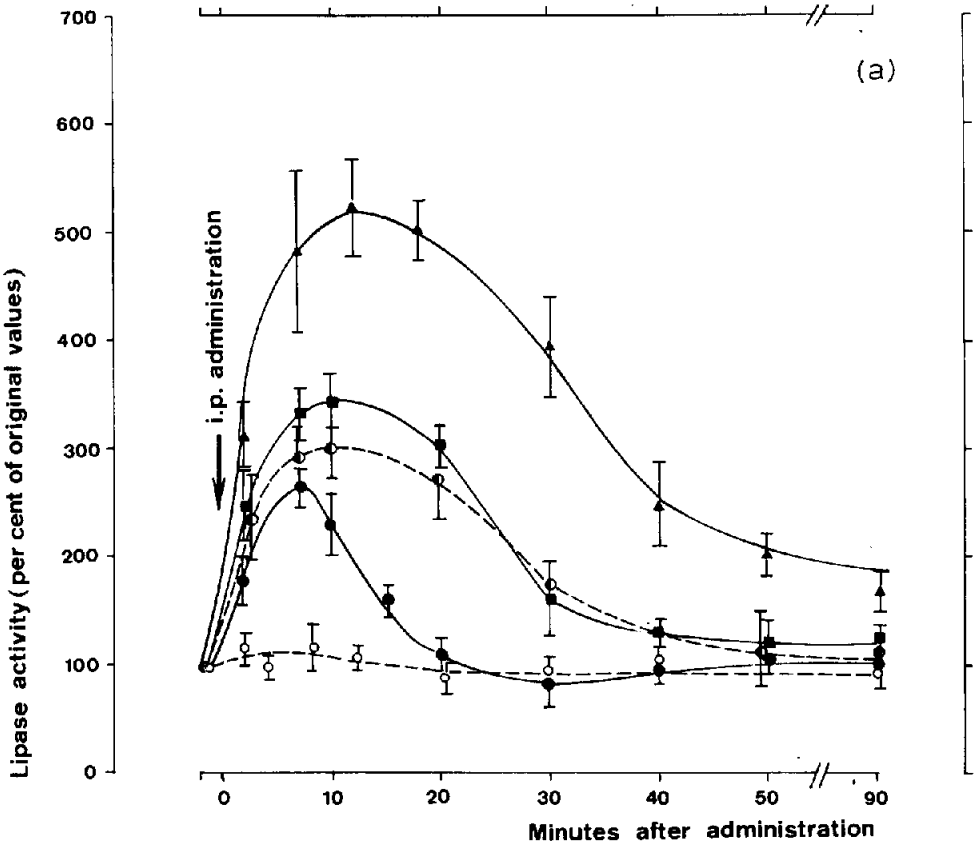

Fig. 1a. Mean change in lipase activity of adipose tissue biopsy samples from zero-time following intraperitoneal administration of saline (o——o——o); DBcAMP (●——●——●, 25 mg/kg, ■——■——■, 50 mg/kg, ▲——▲——▲, 100 mg/kg) or norepinephrine (●—●—●, 5 μmoles/kg). Vertical lines represent standard error. Substrate 2-naphthol laurate 0.5 mM. When nicotinic acid was injected instead of insulin in the dose of 100 mg/kg with DBcAMP or norepinephrine, the results did not differ significantly from those obtained with insulin.

5×10^{-6} M ATP, 1 mM dithiothreitol, 2 mM Mg acetate, 10^{-6} M cAMP and purified heart muscle protein kinase (60 μg in total volume 0.2 ml). The emulsion of substrate was added at the end of the preincubation in a volume of 1 ml and the incubation continued at 37°C for 20 min.

Lipase activity was determined as described elsewhere [10] using either 2-naphthol laurate or tri-, di- or monoolein, trilaurin as substrate or endogenous substrate (prepared according to Vaughan et al. [11]). The preparation of emulsions of other substrates used has been described previously [10]. Lipase activity was measured by the amount of 2-naphthol liberated from 2-naphthol laurate estimated by the slightly modified method of Nachlas and Seligman [12] or FFA liberated from glycerides estimated by the method of Duncombe [13]. Protein content was measured by the method of Lowry et al. [14].

The drugs employed in this study were: $N^6$2′-*O*-dibutyryl adenosine 3′5′-monophosphate (DBcAMP) as the sodium salt (Boehringer, Tutzing, Germany), D(−)norepinephrine (Farbwerke Hoechst AG, Frankfurt, Germany) and Numal (Hoffmann-La Roche). The substrates were from Fluka AG, Buchs, Switzerland. 2-Naphthol laurate was 99.9% pure, the glycerides were purified by thin layer chromatography on silica gel (*n*-heptan–diisopropyl ether–acetic acid 6:4:0.2 v/v/v) to yield a single spot in iodine vapour [15].

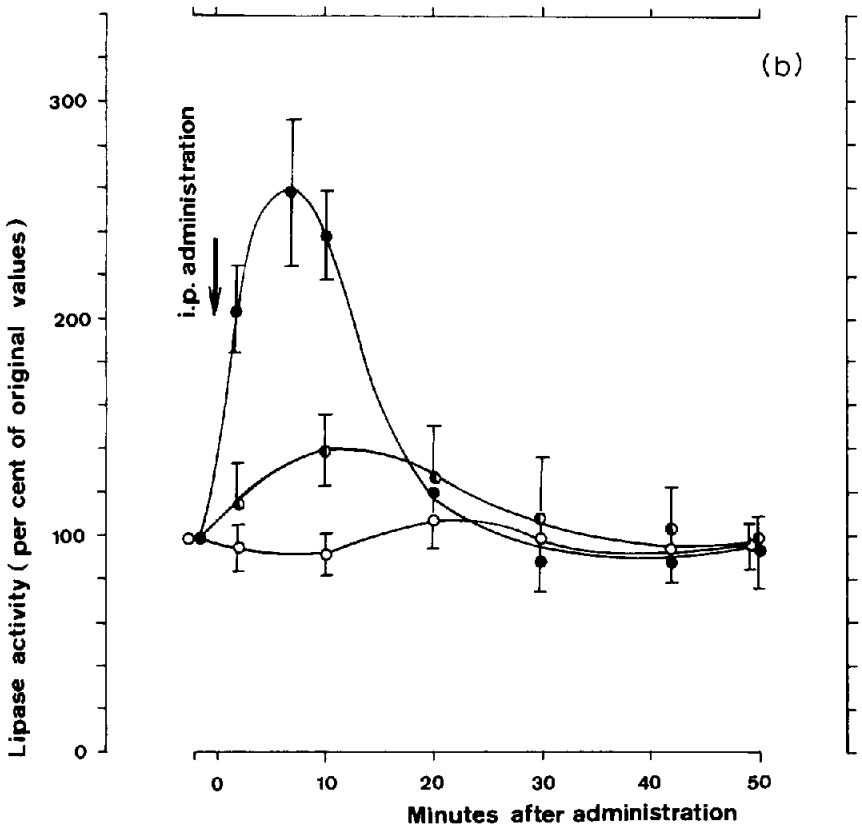

Fig. 1b. Mean change in lipase activity of adipose tissue biopsy samples from zero-time following intraperitoneal administration of insulin, 0.05 U/kg (o—o—o) simultaneously with norepinephrine 5 μmoles/kg (•—•—•) or with DBcAMP, 25 mg/kg (•—•—•). For further explanation, see legend of fig. 1a.

Protein kinase was purified from beef heart by the method of Miyamoto et al. [16] through the DEAE-cellulose column step and stored at −70°C. Preparations were thawed immediately prior to use.

3. Results and discussion

In fig. 1 the time–course of changes in lipase activity of adipose tissue biopsy samples is shown, after the i.p. injection of saline, DBcAMP or norepinephrine with or without insulin. The injections of both lipolytic agents were followed by a significant but transient elevation of adipose tissue lipase activity (fig. 1a), the percentage and duration of activation was dependent on the dose of drug used. Insulin as well as nicotinic acid when injected simultaneously with norepinephrine inhibited its lipolytic effect, whereas the stimulation of adipose tissue lipase activity by DBcAMP failed to be influenced by any of the anti-lipolytic agents used.

In these studies we measured the lipase activity of the enzyme preparation by the cleavage of 2-naphthol laurate. Cleavage of this substrate was shown to parallel that of purified triglycerides by the hormone-sensitive lipase [10] while representing a more convenient measurement of lipolytic activity (2-naphthol laurate was split at a rate about 50 times higher than triolein). From the results documented in table 1 it is obvious that the changes in 2-naphthol laurate cleavage after the administration of DBcAMP or norepinephrine correlated well with the changes of trilaurin and triolein cleavage whereas the hydrolysis of monoolein was not affected.

The action of insulin or nicotinic acid, which both reduced the activity of norepinephrine, but not that

Table 1

Lipase acitivity of homogenates of adipose tissue samples taken before the injection, at the peak effect and 30 min after the peak effect of DBcAMP (25 mg/kg body weight i.p.) or norepinephrine (5 μmoles/kg body weight i.p.). Comparison of different substrates (concentrations insuring maximal rates were used).

Drug injected	Substrate	Lipase activity (nmoles 2-naphthol or FFA/min/mg protein)		
		Before the injection of the respective drug	At the peak effect	30 min after the peak
DBcAMP 25 mg/kg	2-Naphthol laurate 0.5 mM	185.0 ± 14.0	425.5 ± 31.6 +)	190.4 ± 17.1 NS
	Trilaurin 40 mM	8.15 ± 0.7	16.45 ± 1.1 +)	6.93 ± 0.7 NS
	Triolein 10 mM	3.55 ± 0.2	15.48 ± 1.2*	4.05 ± 0.32 NS
	Monoolein 20 mM	23.8 ± 2.0	25.5 ± 2.4 NS	24.9 ± 1.9 NS
	Endogenous substrate	3.36 ± 0.3	5.6 ± 0.41 +)	2.94 ± 0.19 NS
Norepinephrine 5 μmoles/kg	2-Naphthol laurate 0.5 mM	179.5 ± 16.1	348.7 ± 29.0 +)	190.5 ± 18.7 NS
	Trilaurin 40 mM	7.8 ± 0.5	14.8 ± 1.1 +)	8.4 ± 0.7 NS
	Triolein 10 mM	3.2 ± 0.3	6.0 ± 0.4 +)	3.4 ± 0.25 NS
	Monoolein 20 mM	25.6 ± 2.3	24.9 ± 2.9 NS	26.1 ± 2.3 NS
	Endogenous substrate	4.0 ± 0.3	7.9 ± 0.6 +)	3.7 ± 0.22 NS

The values represent the mean ± S.E. from 5 experiments. Statistical significance calculated against control values (before the injection).

+) $p < 0.01$.

* $p < 0.001$.

NS, not significant.

of DBcAMP (fig. 1b), confirm that under these in vivo conditions we are dealing with the same phenomenon observed in vitro [cf. 1]. When samples of adipose tissue were taken before the administration of DBcAMP or norepinephrine, at the peak of their effects or 30 min later when the lipolytic effect had already disappeared and tested for responsiveness to further addition of cAMP, the samples taken before administra-

Table 2

Activation of lipase activity by cAMP in vitro in adipose tissue homogenates obtained before the injection, at the peak effect and 30 min after the peak effect of DBcAMP (25 mg/kg body weight) or norepinephrine (5 μmoles/kg body weight). For preincubation medium see Materials and methods. Lipase substrate was 0.5 mM 2-naphthol laurate. The results represent the mean ± S.E. from 6 experiments.

Preincubation medium	Lipase activity (nmoles 2-naphthol/min/mg protein)					
	Tissue samples from norepinephrine treated rats			Tissue samples from DBcAMP treated rats		
	Before the injection	At the peak effect	30 min after peak effect	Before the injection	At the peak effect	30 min after peak effect
Complete system	413 ± 35.1*	455 ± 41.2 NS	392 ± 32.9*	389 ± 30.1 ‡	410 ± 39.2 NS	401 ± 35.3*
cAMP omitted	182 ± 16.0	479 ± 43.1	170 ± 15.2	165 ± 13.3	422 ± 37.5	176 ± 15.9
Protein kinase omitted	238 ± 21.1 +)	432 ± 37.4 NS	222 ± 20.2 +)	221 ± 19.0 +)	413 ± 39.3 NS	209 ± 18.7 NS

Statistical significance calculated against appropriate control values (cAMP omitted).

+) $p < 0.05$.

* $p < 0.01$.

‡ $p < 0.001$.

NS, not significant.

tion of drugs or after their effect had disappeared, both responded by an increase of lipase activity averaging 100%. The results (table 2) provide strong evidence that the primary action of DBcAMP as well as of norepinephrine consists of the stimulation of triglyceride lipase(s) in adipose tissue. The fact that Bieck et al. [6] obtained only slight stimulation of adipose tissue lipase activity at an extreme dose of DBcAMP (100 mg/kg body weight) may be related to the fact that their adipose tissue samples were taken 40 min after the injection of DBcAMP, that is about 30 min after the peak effect as observed in the experiments of fig. 1a.

It is known that DBcAMP injection results in the elevation of circulating insulin [18]. The fact that insulin administered simultaneously with DBcAMP did not result in the inhibition of adipose tissue lipase activity suggest that this hormone is not directly responsible for the observed decrease in plasma FFA and glycerol level following the injection of DBcAMP [6]. These observations are in agreement with those of Hepp [19] and Hall and Ball [20] who did not find any effect of insulin on the DBcAMP stimulated lipolysis in adipose tissue in vitro. Our results (fig. 1b) support the idea that insulin physiologically acts rather on cAMP formation than on its further effect in lipase activating system.

Acknowledgements

The excellent technical assistance of Miss A.G. Lemmens and the helpful discussions and revision of the manuscript by Professor G. Sharp and Professor E. Shafrir are greatly acknowledged. The study was supported in part by grant No. 3.289.69 from F.N.S.R.S.

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