tal samples. To inhibit the aggregation of blood platelets at the beginning of the experiments DHE (Dihytamin®, VEB Arzneimittelwerk Dresden, German Democratic Republic) or BOL 148 (bromolysergic acid diethyl amide, Forschungsabteilung Sandoz AG, Basel, Switzerland) at concentrations of 10^{-11} M and 3×10^{-6} M, resp., were added.

Addition of [^{14}C]adrenaline results in aggregation of blood platelets which reaches a maximum value within 3–5 min. The uptake of adrenaline by the blood platelets starts immediately after the addition and, although the platelets are aggregated, it proceeds at the same velocity (Figs. 1a and b). In additional experiments adrenaline uptake was measured over a longer incubation time (180 min) under similar conditions but without stirring the suspensions. A linear increase in adrenaline radioactivity in the blood platelets was found. In 10 rabbits the uptake amounted up to 267 ± 197 dis/min per 10^9 blood platelets per min (mean ± standard deviation). This value corresponds to $2{\cdot}42 \pm 1{\cdot}79$ pmoles [^{14}C]adrenaline /10^9 blood platelets per min. The considerable spreading of the values reflects remarkable individual differences between the uptake capacities for adrenaline of the blood platelets in the several animals.

While adrenaline-induced aggregation of blood platelets is completely inhibited under the influence of DHE and BOL 148 (Figs. 1a and b), there are no significant differences in [^{14}C]adrenaline uptake of the experimental samples compared with that of non-treated controls. The apparent increase in adrenaline uptake after 10 min in the presence of DHE does not show statistical significance.

The present data do not favour the assumption that the adrenaline-induced aggregation of rabbit blood platelets is caused by the uptake of this amine. The binding of [^{14}C]adrenaline to the platelets is unchanged, while the aggregation is completely blocked by both drugs. From the adrenaline radioactivity found in the blood platelets at the time of maximum aggregation it can be concluded that no more than 15000 to 30000 molecules of adrenaline are bound to one blood platelet.

The competitive nature of the inhibition of adrenaline-induced aggregation by DHE[1] allows the conclusion that adrenaline and DHE compete for a common, at this time only hypothetical, receptor at the platelet surface which is responsible for the aggregation of blood platelets by adrenaline. Since during incubation with 10^{-11} M DHE only 15 molecules of inhibitor are available for each platelet, the possibility of inhibiting the aggregation by a few molecules of DHE reflects the extraordinarily strong affinity between this drug and the adrenaline receptor and allows the conclusion that the amount of adrenaline necessary for the induction of aggregation is extremely small.

The linear increase in adrenaline radioactivity in blood platelets observed after prolonged incubation is in accordance with the findings of Born and Smith[10] that adrenaline uptake of human blood platelets, at least at adrenaline concentrations up to 10^{-4} M in the incubation medium, does not show saturation kinetics. In this way, adrenaline uptake differs substantially from serotonin uptake. BOL 148 inhibits adrenaline-induced aggregation of blood platelets only at higher concentrations and non-competitively[1]. Contrary to DHE, it seems to influence adrenaline-induced aggregation by a rather unspecific mechanism. From data obtained with human blood platelets it is apparent that BOL 148 acts by influencing the second phase of adrenaline-induced aggregation representing the release reaction of endogenous ADP* which triggers the further aggregation of blood platelets[11, 12].

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Effects of *O*-2′ and N^6-acyl-substituted cyclic AMP on adipose tissue metabolism *in vitro*

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Cyclic AMP (cAMP) mediates in many of the actions of a variety of hormones[1]. Following beta-adrenergic stimulation, fat cell adenyl cyclase promotes formation of cAMP; this allosteric effector[2] binds to protein kinase which then promotes activation of hormone-sensitive lipase resulting in glycerol and free fatty acid (FFA) production. The dibutyryl derivative of cAMP (dbcAMP) is a well-known potent lipolytic agent in adipose cells incubated *in vitro* (e.g. ref. 4). Its high potency is partly attributable to its lipophyllic nature allowing rapid penetration of fat cell membranes[5], and partly to resistance to phosphodiesterase activity[6]. Solomon *et*

al.[5] showed that whereas dbcAMP enhances fat cell lipolysis, it inhibits glucose utilization by fat cells. This dual effect limits its usefulness as a biochemical tool. A search has therefore been made for other derivatives of cAMP which likewise possess the properties of rapid uptake by cells, and insensitivity to endogenous phosphodiesterases.

The substances investigated were cAMP, dbcAMP, N^6-monobutyryl cyclic AMP (N^6-mbcAMP), *O*-2′-monosuccinyl cyclic AMP (*O*-2′-scAMP), *O*-2′-monobutyryl cyclic AMP (*O*-2′-mbcAMP) and noradrenaline, these were obtained from The Boehringer Corporation (London) Ltd. [U-^{14}C]glucose was purchased from the Radiochemical Centre, Amersham. They were examined for their effects on lipolysis and total lipid biosynthesis in fat cells obtained from rats (Olac, Oxford) (a) allowed food and water *ad lib.* or (b) fasted but permitted water *ab lib.*

Epididymal adipose tissue was used to prepare suspensions of isolated fat cells in Krebs–Ringer bicarbonate buffer as described previously[4, 7].

Initially the lipolytic potencies of cAMP, dbcAMP and *O*-2′-scAMP were compared at concentrations ranged between 2×10^{-5} M and 2×10^{-3} M. Further studies were then undertaken to compare the lipolytic activities of dbcAMP, N^6-mbcAMP, *O*-2′mbcAMP and *O*-2′-scAMP at a single concentration of 10^{-3} M, with noradrenaline at 10^{-4} M.

Glycerol release was employed as the index of lipolysis[8] which is expressed as nmoles glycerol released per mg fat cell lipid during 90 min incubation. Total lipid was determined by a technique[9] modified from Folch, Lees and Sloan-Stanley[10].

Total lipid biosynthesis was estimated by measuring the incorporation of tracer amounts of [U-^{14}C]glucose. Lipid was extracted from the fat cells using chloroform–methanol, an aliquot evaporated and the lipid residue taken up in toluene phosphor, as described previously[9].

Effects of cAMP and related compounds on glycerol release

Comparative results from two experiments are shown in Table 1. Strong lipolytic effects were shown by cAMP, dbcAMP, N^6-mbcAMP and noradrenaline but the *O*-2′-acyl derivatives *O*-2′-scAMP and *O*-2′-mbcAMP were inactive. These results confirm the powerful effect of dbcAMP and emphasize that its effect increased more sharply with concentration than that of cAMP. In general, effects (including control release) in fasted rats exceeded those in fed rats. In fat cells from fed rats the effects were less with both cAMP and dbcAMP and again *O*-2′-scAMP was inactive.

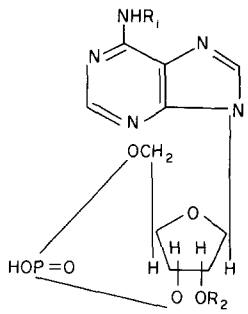

Fig. 1. Basic cyclic nucleotide structural formula of the acyl-substituted derivatives examined for their effects on fat cell metabolism *in vitro*.

Compound	R_1	R_2
1. Adenosine 3′5′-cyclic monophosphate.	H	H
2. N^6-monobutyryl adenosine 3′,5′-cyclic monophosphate (N^6-mbcAMP).	COPr	H
3. N^6-*O*-2′-dibutyryl adenosine 3′,5′-cyclic monophosphate (dbcAMP).	COPr	COPr
4. *O*-2′-monosuccinyl adenosine 3′,5′-monophosphate (*O*-2′-scAMP).	H	$CO(CH_2)_2$
5. *O*-2′-monobutyryl adenosine 3′,5′-cyclic monophosphate (*O*-2′-mbcAMP).	H	COPr

Table 1. Effects of cAMP, dbcAMP, *O*-2′-scAMP, N^6-mbcAMP, *O*-2′-mbcAMP, and noradrenaline on glycerol release from rat isolated fat cells.

Compound added	Molar concn $\times 10^{-5}$	Fat cells from starved rats		Fat cells from fed rats	
		Expt. 1	Expt. 2	Expt. 1	Expt. 2
Control	—	3·5 ± 0·1	2·6 ± 0·1	2·8 ± 0·2	2·4 ± 0·1
cAMP	2	6·6 ± 1·1*	—	2·5 ± 0·2	—
	20	6·8 ± 1·2	—	2·1 ± 0·2	—
	200	9·7 ± 0·3‡	—	4·1 ± 0·2*	—
dbcAMP	2	3·5 ± 0·2	—	2·3 ± 0·3	—
	20	22·4 ± 0·6‡	—	4·9 ± 0·3†	—
	100	—	34·6 ± 0·9‡	—	43·5 ± 1·2‡
	200	41·1 ± 3·9‡	—	44·2 ± 1·8‡	—
O-2′-scAMP	2	4·1 ± 1·3	—	1·9 ± 0·4	—
	20	3·5 ± 0·4	—	1·9 ± 0·4	—
	100	—	2·5 ± 0·1	—	2·2 ± 0·1
	200	3·7 ± 0·5	—	2·7 ± 0·4	—
N^6-mbcAMP	100	—	34·1 ± 2·2‡	—	28·3 ± 1·6‡
O-2′-mbcAMP	100	—	2·6 ± 0·1	—	2·6 ± 0·3
Noradrenaline	10	—	33·3 ± 1·1‡	—	23·5 ± 0·5‡

Data are nmoles glycerol released/mg lipid per 90 min incubation, mean of 3 observations ±S.E.M. Significance of difference from respective control values ‡ $P < 0.001$, † $P < 0.01$, * $P < 0.05$.

Table 2. Effects of dbcAMP, N^6-mbcAMP, O-2′-mbcAMP, O-2′-scAMP and noradrenaline on the incorporation of [U-^{14}C]glucose into fat cell lipid

Source of fat cells	Lipogenic agent (cAMP derivatives used at 10^{-3} M and noradrenaline at 10^{-4} M)					
	Control	Noradrenaline	dbcAMP	N^6-mbcAMP	O-2′-mbcAMP	O-2′-scAMP
Fed rats	1349 ± 55	4028 ± 266‡	628 ± 32‡	1078 ± 33*	1421 ± 15	1499 ± 120
Starved rats	1002 ± 89	4430 ± 328‡	540 ± 19‡	823 ± 123	1096 ± 56	1061 ± 110

Data are c.p.m. per 50 mg of fat cell lipid, mean of 3 observations ±S.E.M. Significance of difference from respective controls: ‡ $P < 0.001$, * $0.01 > P < 0.05$.

Effects of the dbcAMP, N^6-mbcAMP, O-2′-mbcAMP, O-2′-scAMP and noradrenaline on the incorporation of [U-^{14}C]glucose into fat cell lipid

Noradrenaline significantly enhanced [U-^{14}C]glucose incorporation into the total lipid of fat cells obtained from both fed and fasted rats, whereas dbcAMP significantly ($P < 0.01$) reduced incorporation in fat cells of both types (Table 2). N^6-mbcAMP behaved in a similar fashion to the dibutyryl derivative but its inhibition of glucose incorporation was not significant in fat cells from fasted rats. Contrarily, the O-2′-acyl derivatives of cAMP did not inhibit total lipid biosynthesis in fat cells obtained from fed or fasted animals. Overall there was a greater incorporation of labelled glucose into the fat cells obtained from fed rather than fasted rats.

The greater lipolytic potency of dbcAMP compared with cAMP corroborates other work[5] and is compatible with rapid penetration of the synthetic agonist into fat cells and low sensitivity to inactivation by intracellular phosphodiesterase[6]. The lack of lipolytic activity of the succinyl derivative was less readily explained.

That N^6-mbcAMP was a potent lipolytic agent whereas O-2′-mbcAMP was not, is compatible with the view that an available O-2′ locus of cAMP is a necessary requirement for a powerful physiological effect. Thus the lipolytic inactivity of the O-2′-scAMP may be attributed to the O-2′-acyl substitution rather than to the succinyl grouping *per se*. Miller *et al.*[11] showed that N^6-mbcAMP stimulates bovine brain or heart protein kinase as efficaceously as cAMP itself whilst O-2′-mbcAMP is far less effective. Further, N^6-mbcAMP is a poor substrate for bovine or rabbit tissue phosphodiesterase whilst O-2′-mbcAMP is a good one. Thus it maybe concluded that O-2′-acyl substituted cyclic AMP derivatives lack lipolytic potency due to their inability to stimulate adipose tissue protein kinase and to their rapid inactivation by phosphodiesterase. N^6-acyl derivatives also inhibit cAMP phosphodiesterase and may enhance lipolysis by causing accumulation of endogenous cyclic AMP. Some of the biological activity of dbcAMP has been ascribed to its conversion to N^6-mbcAMP (ref. 12), and Miller *et al.*[11] have also suggested that N^6-mbcAMP is the active form of dbcAMP. The contribution of this phenomenon to the lipolytic activity of dbcAMP remains to be elucidated. However, it is known that the O-2′-acyl bond is a very labile linkage and will hydrolyse at alkaline pH in aqueous solution[13]. This indicates that effects of dbcAMP may well be due to N^6-mbcAMP and, further, that the lack of O-2′-acyl compounds could be due to their conversion to 3′, 5′-cyclic AMP which will not penetrate fat cells.

The conspicuous effect of noradrenaline in enhancing total fat cell lipid biosynthesis is possibly explained by stimulation of the incorporation of [U-^{14}C]glucose into glyceride glycerol[14].

The inhibition of [U-^{14}C]glucose incorporation into fat cell lipid by dbcAMP and N^6-mbcAMP is in general agreement with the work of Solomon *et al.*[5]. However, it has been shown that dbcAMP at low concentrations (1×10^{-4}–5×10^{-4} M) may increase labelled glucose incorporation into the lipid of fragments of adipose tissue, but not when at higher concentrations (3×10^{-3}–5×10^{-3} M) [15].

The absence of inhibition of total lipid synthesis by the O-2′-acyl-substituted derivatives of cyclic AMP is an interesting finding. Whereas in the case of lipolysis the N^6-acyl substituted nucleotides were active and O-2′-acyl ones were not, in the case of fat synthesis the N^6-acyl substituted derivatives actively inhibited whilst O-2′-acyl derivatives did not. This is compatible with hypothesis that the O-2′ locus of cAMP should be available for reaction during lipolysis and that perhaps an available N^6 locus is a requirement for fat synthesis.

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