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Influence of acetaldehyde on stimulated lipolysis and cyclic AMP formation in isolated rat fat cells. Comparison with ethanol

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A few years ago, we showed that acetaldehyde (AcH) [1], but not ethanol [2] increases the basal lipolytic activity of rat adipose tissue *in vitro*. We concluded that this effect was not mediated by cyclic AMP (cAMP) but was related to inhibition of glycolysis and respiration in adipose tissue [1]. In recent reports, we found that the thiol-blocking agent *N*-ethylmaleimide (NEM) stimulates, like AcH, the basal rate of lipolysis in both rat [3] and human [4] fat cells. Paradoxically, NEM and other thiol-blocking agents were also shown to inhibit the lipolytic action of catecholamines, theophylline and dibutyryl cyclic AMP (Dc AMP) [4–6]. Because of the thiol-blocking ability of aldehydes [7], we were led to question whether AcH may also inhibit the stimulated lipolytic activity in isolated fat cells.

This was the aim of the present study in which we investigated the influence of different concentrations of AcH on rat fat cell lipolysis and cAMP accumulation stimulated by different drugs. Furthermore, as AcH is the primary metabolite of ethanol and since controversial data have been published on the effects of ethanol on cAMP metabolism in different tissues [8–10], the influence of ethanol on these processes was also investigated.

The present results clearly show that while ethanol has no effect on both stimulated lipolysis and cAMP accumulation, AcH, on the contrary, induces a dose-dependent inhibition of both processes. The mechanism and the significance *in vivo* of this inhibition are discussed.

Fat cells were isolated by collagenase digestion of epididymal fat pads removed from 24 hr fasted Wistar rats weighing 220–250 g, essentially as described previously [11]. Aliquots (equivalent to 40 ± 10 mg cell lipid or 2.5 to 4×10^5 cells) of the isolated washed fat cells were transferred into plastic flasks containing Krebs–Ringer bicarbonate buffer (pH 7.4), calcium (1.27 mM), glucose (5 mM) and albumin at different concentrations (2% when cAMP was assayed and 4% when lipolysis was determined), the final volume being 2 ml. Incubations were performed for either 5 min (determination of cAMP) or 60 min (determination of lipolysis) in a shaking water bath at 37°. Unless otherwise stated, all agonists and antagonists tested were added at the start of the incubation. Because of the high volatility of AcH, the plastic flasks were stoppered during the incubations. Following this procedure, we have previously found that the amount of AcH which disappeared from the medium after 3 hr incubation accounted for less than 10 per cent of the concentration added and was due to fat cell consumption [1, 2]. Reactions were terminated by separating the fat cells from the medium by a rapid centrifugation at +4° [11] and lipolysis (glycerol release) was determined and expressed as previously described [12].

Cyclic AMP present in the fat cells or released into the medium was extracted and determined according to a slight modification of the radiocompetitive assay of Brown *et al.* [13] as previously described [12]. Interference of albumin in the assays of cAMP released into the medium [14] was avoided by using blanks and standards prepared with aliquots of the incubation buffer. Overall recovery of cAMP was between 90 and 95 per cent.

Metabolic data are expressed on the basis of fat cell lipid dry wt [11]. Results are given as mean values ± S.E. and Student's "*t*" test was used for comparison of mean values.

Acetaldehyde and ethanol were from Merck, cAMP, [^{3}H]cAMP (sp. act. 25 Ci/m-mole) and cAMP binding protein from the Radiochemical Centre Amersham, U.K., norepinephrine and DcAMP from Sigma and enzymes, coenzymes and substrates from Boehringer. All other chemicals were of analytical grade.

The basal content of cAMP in fat cells was about 35–55 pmoles/g cell lipid (or 4–6 pmoles/10^6 cells). It was unaffected by AcH concentrations ranging from 10^{-5} to 10^{-3} M but was significantly depressed (50 per cent) by the concentration of 10^{-2} M (data not shown) i.e. the concentration of AcH producing the maximal stimulation of basal lipolysis [1]. This result therefore give an additional support to our previous hypothesis according to which an increase in cAMP formation is not involved in the lipolytic action of AcH [1].

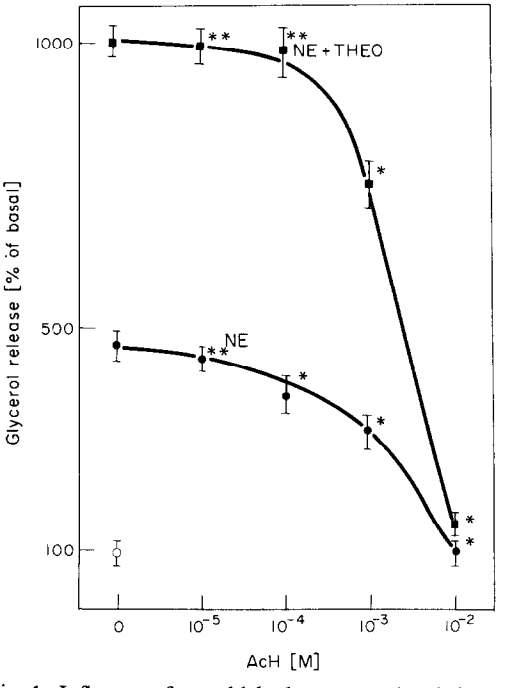

Fig. 1. Influence of acetaldehyde on norepinephrine—or on norepinephrine plus theophylline stimulated lipolysis in rat fat cells. Fat cells were incubated in the absence (basal) or in the presence of 10^{-6} M norepinephrine (NE) or 10^{-6} M norepinephrine + 5×10^{-3} M theophylline (NE plus THEO) with or without different concentrations of acetaldehyde (AcH). After one hour incubation, lipolysis was determined. Results are expressed as per cent of the basal lipolytic activity (open circle) which was 5.8 ± 0.7 μmoles glycerol release/g cell lipid/hr. Each point is the mean ± 2 S.E. (vertical lines) of four to five determinations.* $P < 0.01$; †$P > 0.05$.

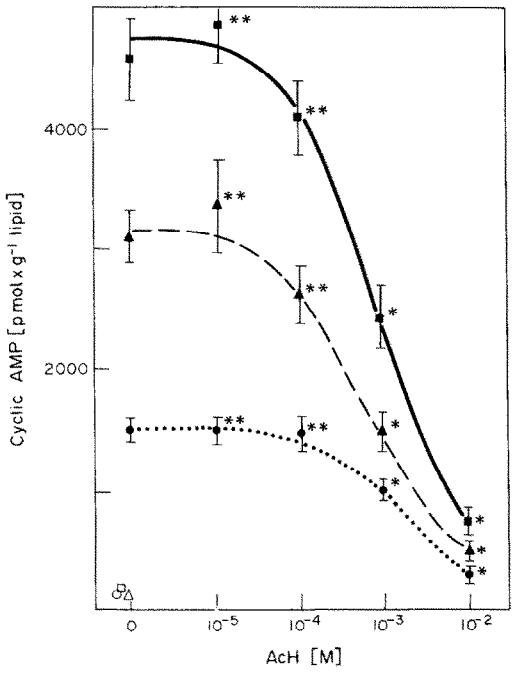

Fig. 2. Influence of acetaldehyde on norepinephrine plus theophylline-induced cyclic AMP accumulation in and release from rat fat cells. Fat cells were incubated in the absence (open symbols) or presence of 10^{-6} M norepinephrine + 5×10^{-3} M theophylline (closed symbols) with or without different concentrations of acetaldehyde (AcH). After 5 min incubation, cAMP was determined in both the fat cells and the medium. ●.....● = cAMP released into the medium; ▲-----▲ = cAMP in the fat cell; ■——■ = total cAMP. Each point is the mean ±2 S.E. (vertical lines) of four to five determinations. *P < 0.01; †P > 0.05.

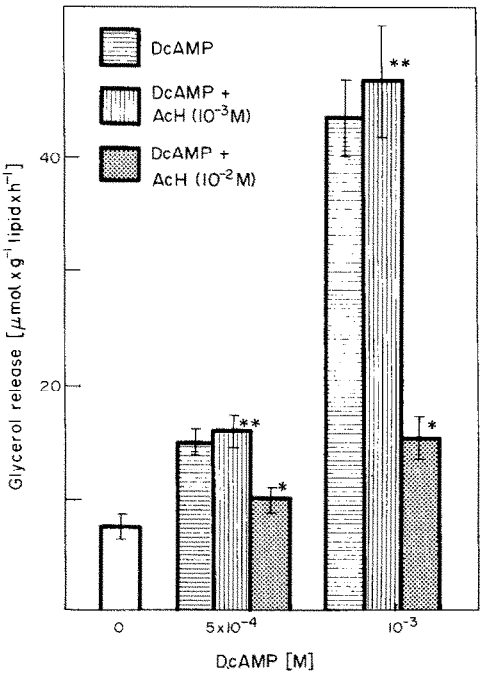

Fig. 3. Influence of acetaldehyde on dibutyryl cyclic AMP-induced lipolysis in rat fat cells. Fat cells were incubated in the absence or in the presence of 5×10^{-4} M or 10^{-3} M dibutyryl cyclic AMP (DcAMP) with or without different concentrations of acetaldehyde (AcH). After 1 hr incubation, lipolysis was determined. Each bar represents the mean ±2 S.E. (vertical lines) of five determinations. *P < 0.01; †P > 0.05.

Addition of 10^{-5} to 10^{-2} M AcH produced a dose-dependent inhibition of norepinephrine (10^{-6} M) stimulated lipolysis, inhibition which was almost complete at the concentration of 10^{-2} M (Fig. 1). When lipolysis was maximally stimulated by the addition of both norepinephrine (10^{-6} M) and theophylline (5.10^{-3} M), a concentration of at least 10^{-3} M of AcH was required to observe an inhibition of the glycerol release (Fig. 1). Under these conditions, AcH induced a parallel dose-dependent decrease in the intracellular cAMP accumulation (Fig. 2). This reduction which was significant at the concentration of 10^{-3} M, was not due to a permissive effect of AcH on the output of cAMP from the cell since a similar reduction occurred in the cAMP released into the medium (Fig. 2). As shown in Fig. 3, lipolysis induced by DcAMP at two different concentrations (5.10^{-4} M and 1.10^{-3} M) was also markedly reduced by 10^{-2} M AcH but not by lower concentrations.

The influence of ethanol (10^{-5} to 10^{-1} M) on both lipolysis and cAMP accumulation was studied. Opposite to AcH, ethanol, even at the concentration of 10^{-1} M failed to affect the rate of lipolysis induced by norepinephrine, norepinephrine plus theophylline and DcAMP and had no significant effect on the catecholamine plus theophylline-induced cAMP accumulation in the fat cell (Table 1). This lack of effect was also found when fat cells were preincubated for 45 min with ethanol alone before the addition of the lipolytic agents (data not shown).

Controversial reports have been published concerning the effects of ethanol on the cAMP formation in the liver. In fact, high concentrations of ethanol were reported to stimulate adenylate cyclase in both liver homogenates and the washed particle enzyme [8], whereas others only found stimulation in plasma membranes but not in homogenates [9]. On the contrary, acute administration of ethanol was shown to reduce the cAMP level in the liver [10] and the synthesis of cAMP from labeled ATP [10]. More recently, Zederman *et al.* [15] reported that ethanol concentrations similar to those presently used markedly inhibited the glucagon-stimulated cAMP formation in intact hepatocytes. This inhibition, which was also induced by lactate, was attributed to the inhibition of adenylate cyclase by the large excess of NADH [16] resulting from ethanol oxidation.

In adipose tissue, extremely high concentrations of ethanol were reported to increase the basal cAMP level [17]. Our present results show that ethanol concentrations similar to those found in the blood after acute ethanol administration [18], not only fail to affect the basal cAMP content but also the catecholamine-induced cAMP accumulation and lipolysis in rat fat cells. This lack of action, which contrasts with the above mentioned effects of ethanol on hepatocytes [15], can be explained by the very low alcohol dehydrogenase activity in fat cells compared with the high activity found in the liver [19].

On the contrary, incubation of fat pads with AcH or with other aliphatic or aromatic aldehydes have been shown to induce a marked increase of the NADH/NAD^+ ratio in the cytosol [1, 20]. Therefore, and because of the inhibitory effect of NADH on adenylate cyclase activity in isolated fat cell membranes [16], it is likely that the presently reported inhibition of catecholamine-induced cAMP formation by AcH is related to the intracellular redox state changes caused in the fat cell by AcH. Evidence in favour of such a mechanism is further-

Table 1. Failure of ethanol to modify lipolysis and cyclic AMP accumulation in rat fat cells

Addition to the medium					Glycerol release (μmoles $\times$ g^{-1} cell lipid $\times$ hr^{-1})	Intracellular cAMP (pmoles $\times$ g^{-1} cell lipid)
Norepinephrine (10^{-6} M)	Theophylline (5×10^{-5} M)	DcAMP (5×10^{-4} M)	DcAMP (10^{-3} M)	Ethanol (10^{-1} M)		
—	—	—	—	—	5.5±0.4	52±8
—	—	—	—	+	6.1±0.5*	45±12*
+	—	—	—	—	28.8±1.9	ND
+	—	—	—	+	24.3±3.2*	ND
+	+	—	—	—	61.2±3.9	2347±281
+	+	—	—	+	53.8±5.4*	1940±175*
—	—	+	—	—	10.8±0.7	ND
—	—	+	—	+	11.1±0.1*	ND
—	—	—	+	—	43.6±3.2	ND
—	—	—	+	+	42.6±1.5*	ND

Fat cells were incubated for 1 hr (determination of glycerol release) or for 5 min (determination of cAMP). Experimental conditions were as described in Figs 1 and 2, except that ethanol was added in place of acetaldehyde.

Each value represents the mean ± S.E. of five determinations.

ND: not determined.

* $P > 0.05$.

more provided by the fact that AcH also reduces the basal cAMP level in the fat cell.

Besides its inhibitory effect on catecholamine-induced lipolysis, an effect which could be explained by its action on cAMP synthesis, AcH also inhibits the lipolytic action of DcAMP. As DcAMP mimicks the effects of endogenous cAMP [21], this led to the conclusion that AcH also inhibits the lipolytic process at a step localized beyond cAMP formation. Further studies will be necessary to determine the site of the latter inhibition (cAMP binding to protein kinase, protein kinase or triglyceride lipase activation).

The part played *in vivo* by the presently reported *in vitro* actions of AcH in the metabolic disturbances following acute ethanol administration is questionable. In fact, the minimal AcH concentration eliciting a significant inhibition of catecholamine-induced lipolysis is about 3–50-fold higher than the AcH levels found in the blood after acute ethanol administration [22]. Furthermore, acute ethanol administration is usually followed by an increased lipolysis [23] which has been attributed either to stress [23] or to a direct stimulatory effect of AcH on the basal lipolytic activity of adipose tissue [1]. On the contrary, the present findings *in vitro* could be of importance in understanding the reduction of lipolysis which occurs *in vivo* after AcH infusion [24], a condition under which a concomitant increase in catecholamine secretion is observed [24].

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