

Alterations in Response of Rat White Adipocytes to Insulin, Noradrenaline, Corticotropin and Glucagon after Adrenalectomy

CORRECTION OF THESE CHANGES BY ADENOSINE DEAMINASE

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1. Adipocytes isolated from rats 6–9 days after adrenalectomy had significantly increased sensitivity to insulin action against noradrenaline-stimulated lipolysis. In the presence of adenosine deaminase there was no significant difference in insulin sensitivity between cells from adrenalectomized and sham-operated rats. 2. Adipocytes from adrenalectomized rats had decreased lipolytic responses to all concentrations of noradrenaline and glucagon tested and a decreased lipolytic response to low but not high concentrations of corticotropin. There was no difference in lipolytic response to theophylline after adrenalectomy. Adenosine deaminase corrected the differences in response to noradrenaline and glucagon resulting from adrenalectomy. 3. In the presence of adenosine deaminase rates of lipolysis, after stimulation by high concentrations of noradrenaline, glucagon, corticotropin or theophylline, were the same in cells from adrenalectomized or sham-operated rats. 4. These findings and previously reported effects of adenosine and adrenalectomy on adipocyte function are discussed. It is proposed that changes in adipocyte hormone responsiveness after adrenalectomy may result from changes in adenosine metabolism or release.

Adrenal glucocorticoids play a well-documented 'permissive' role in the maintenance *in vivo* of a normal lipolytic response by adipose tissues (Shafrir & Steinberg, 1960; Shafrir *et al.*, 1960; Maickel *et al.*, 1967). *In vitro* also, it has been observed that the lipolytic response after stimulation by adrenaline, noradrenaline or corticotropin is decreased in adipose tissues or adipocytes isolated from adrenalectomized rats (Schotz *et al.*, 1959; Schönhöfer *et al.*, 1968; Allen & Beck, 1972; Skidmore *et al.*, 1972). This lesion (or lesions) can be corrected by administration of glucocorticoids *in vivo* (Reshef & Shapiro, 1960; Maickel *et al.*, 1967; Schönhöfer *et al.*, 1968) or *in vitro* (Corbin & Park, 1969). Further, the uptake and metabolism of glucose is increased in adipose tissue from adrenalectomized rats (Fain, 1962; Shafrir & Kerpel, 1964). After glucocorticoid administration the reverse is seen, i.e. glucose metabolism is decreased (Munck, 1962; Fain, 1962; Leboeuf *et al.*, 1962; Yorke, 1967; Livingston & Lockwood, 1975), and, in addition, the sensitivity of glucose metabolism to insulin is decreased (Correa *et al.*, 1960; Lundquist, 1968; Olefsky, 1975).

Adenosine, various derivatives of adenosine and some adenine nucleotides or dinucleotides mimic both the antilipolytic action of insulin and insulin stimulation of glucose metabolism by adipose tissue

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in vitro (Dole, 1961, 1962; Kappeler, 1966; Pereira & Holland, 1966; Blackard & Cameron, 1967; Davies, 1968; Fain *et al.*, 1972; Schwabe *et al.*, 1973, 1975). Further, in rat adipocytes, adenosine and its derivatives at low concentrations facilitate the actions of insulin to decrease lipolysis, to stimulate glucose metabolism and to diminish 3':5'-cyclic AMP accumulation (Fain, 1973; Ebert & Schwabe, 1973; Schwabe *et al.*, 1974; Fain & Wieser, 1975; Wieser & Fain, 1975; Schwabe *et al.*, 1975). Preparations of rat adipocytes appear to release small amounts of endogenous adenosine, which can exert the above effects *in vitro* (Schwabe *et al.*, 1973). Addition of adenosine deaminase to incubations prevents these adenosine-dependent effects (Schwabe & Ebert, 1974; Fain & Wieser, 1975).

Smith & Saggerson (1977) found that the antilipolytic response of rat epididymal adipocytes to high concentrations of insulin was altered by adrenalectomy. The present study is an extension of the investigation of this phenomenon. We find that noradrenaline-stimulated lipolysis is more sensitive to insulin in adipocytes from adrenalectomized rats and that addition of adenosine deaminase to these incubations not only abolishes this effect of adrenalectomy but also restores to normal the diminished lipolytic response to noradrenaline, corticotropin and glucagon that is seen in these cells.

These findings suggest that altered production of, metabolism of or sensitivity to adenosine may be

responsible for these changes in adipocyte hormone responsiveness that occur after adrenalectomy.

Materials and Methods

Chemicals

Nucleotides and collagenase (type I from *Clostridium histolyticum*) were obtained from International Enzymes Ltd. (Windsor, Berks., U.K.). Other enzymes and phosphoenolpyruvate were purchased from Boehringer Corporation (London) Ltd. (Lewes, Sussex, U.K.). L-Noradrenaline bitartrate, pig corticotropin (grade II), DNA (type V, sodium salt, highly polymerized; from calf thymus) and theophylline (1,3-dimethylxanthine) were from Sigma (London) Chemical Co. (Kingston upon Thames, Surrey, U.K.). Bovine plasma albumin powder (fraction V) was also from Sigma and was subjected to a defatting procedure (Chen, 1967) with minor modifications described by Saggerson (1972). Bovine glucagon was from Calbiochem Ltd. (Hereford, U.K.) and bovine insulin (six times recrystallized) from Boots Pure Drug Co. Ltd. (Nottingham, U.K.).

Animals

Adrenalectomized and sham-adrenalectomized male Wistar rats were purchased from Charles River (Margate, Kent, U.K.) 1 day after operation at 120–130 g body weight. These were maintained on diet GR3EK (E. Dixon and Sons, Ware, Herts., U.K.) until death 6–9 days after operation. Adrenalectomized rats were given 0.9% (w/v) NaCl solution in tap water to drink. At death sham-operated animals generally weighed 190–200 g and adrenalectomized animals 170–180 g. Fat-pads from sham-operated rats were in the weight range 0.4–0.6 g. Those from adrenalectomized animals were generally 0.3–0.5 g.

Preparation of adipocytes

On each occasion that cells were prepared from adrenalectomized rats a parallel preparation was made from sham-operated animals. The pooled epididymal fat-pads of two rats were disaggregated with collagenase as described by Rodbell (1964). The cells were washed twice with Krebs–Ringer bicarbonate buffer (Krebs & Henseleit, 1932) containing fatty acid-poor albumin (10 mg/ml), then suspended in a 10 ml volume of the same medium. Portions of this stock suspension were then used for incubation or for DNA estimation.

Incubation of adipocytes

Portions (0.5 ml) of the stock cell suspension were added to 25 ml silicon-treated flasks and incubated

with shaking for 1 h at 37°C in a final volume of 4 ml of Krebs–Ringer bicarbonate buffer containing 1.27 mM-Ca²⁺, fatty acid-poor albumin (36 mg/ml) and 5 mM-glucose. The flask contents were continuously gassed with O₂/CO₂ (19:1). Other additions to flasks are indicated in the legends to individual Figures. In any experiment, flasks contained very similar quantities of cells (assessed on the basis of DNA) from adrenalectomized and sham-operated rats.

Analytical methods

Immediately after incubation, flasks were plunged into ice and the contents deproteinized by addition of 0.5 ml of ice-cold 45% (w/v) HClO₄. The mixtures were centrifuged, 0.5 ml of triethanolamine hydrochloride was added to the supernatants and these were then neutralized with conc. K₂CO₃. These extracts were assayed for glycerol by the method of Garland & Randle (1962). Samples of adenosine deaminase (EC 3.5.4.4) were standardized spectrophotometrically by the method of Kalckar (1947); 1 unit is the amount of enzyme needed to convert 1 μmol of substrate/s at 25°C.

The DNA content of 2.0 ml portions of the stock adipocyte suspension was determined as described by Saggerson (1972).

Statistical methods

Statistical significance was determined by Student's *t* test.

Results and Discussion

Effect of adrenalectomy on the antilipolytic action of insulin

Adipocytes from adrenalectomized or sham-operated rats were incubated with 0.25 μM-noradrenaline and the effect of insulin to oppose the resulting lipolysis was examined (Fig. 1). This concentration of noradrenaline was arbitrarily chosen as one giving extensive but yet submaximal stimulation of lipolysis (see Fig. 4). The absolute rate of lipolysis was lower in cells from adrenalectomized rats than in those from sham-operated controls at all noradrenaline concentrations tested (see Fig. 5). Fig. 1 shows that in percentage terms, cells from adrenalectomized rats were more sensitive to the antilipolytic action of insulin. The percentage antilipolytic response was significantly greater ($P < 0.05$, < 0.01 , < 0.05 respectively) at 5, 10 and 20 μunits of insulin/ml (1 unit is 41.7 μg). Smith & Saggerson (1977) found that adipocytes from adrenalectomized rats showed less antilipolytic response to high con-

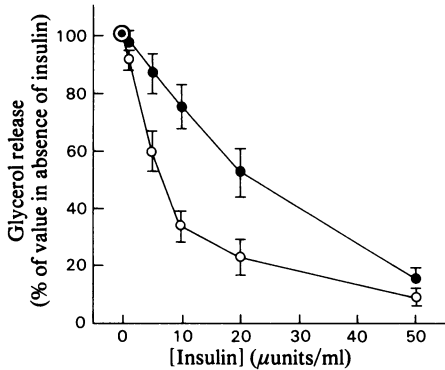


Fig. 1. Antilipolytic effect of insulin on adipocytes from adrenalectomized and sham-operated rats

Adipocytes from adrenalectomized (○) or sham-operated (●) rats were incubated for 1 h with 0.25 μ M-noradrenaline and the indicated concentrations of insulin. The results are means of six independent measurements and the bars indicate S.E.M. The rates of lipolysis in the absence of insulin were 8.5 and 13.4 μ mol of glycerol/h per 100 μ g of DNA for cells from adrenalectomized and sham-operated rats respectively.

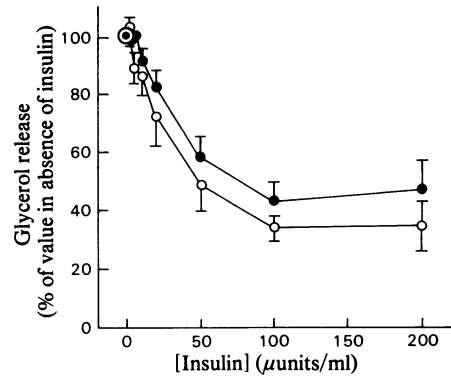


Fig. 2. Antilipolytic effects of insulin on adipocytes from adrenalectomized and sham-operated rats in the presence of adenosine deaminase

Adipocytes from adrenalectomized (○) or sham-operated (●) rats were incubated for 1 h with 8 nM-noradrenaline, adenosine deaminase (50 munits/ml) and the indicated concentrations of insulin. The results are means of six independent measurements and the bars indicate S.E.M. The rates of lipolysis in the absence of insulin were 13.7 and 16.1 μ mol of glycerol/h per 100 μ g of DNA for cells from adrenalectomized and sham-operated rats respectively.

centrations of insulin (20 munits/ml) compared with cells from sham-operated animals. In the present study adrenalectomy led to an increase in sensitivity to low concentrations of insulin. These two sets of observations need not be contradictory, since antilipolytic dose responses for insulin are biphasic (Hales *et al.*, 1968; Lavis *et al.*, 1970; Solomon *et al.*, 1970; Schönhöfer *et al.*, 1972; Kono & Barham, 1973). Both the present and previous observations (Smith & Saggerson, 1977) would be compatible with there being a shift in this biphasic dose response after adrenalectomy. Glucocorticoids have been reported to decrease the sensitivity of adipose-tissue glucose metabolism to low concentrations of insulin (Correa *et al.*, 1960; Lundquist, 1968; Olefsky, 1975). However, we are unaware of any previous report showing increased sensitivity of this tissue after adrenalectomy to the antilipolytic action of insulin. In this respect it is noteworthy that the sensitivity of rat adipose-tissue pieces to an antilipolytic action of calcitonin (against basal lipolysis) is increased after adrenalectomy and decreased by glucocorticoid administration (Werner & Löw, 1974).

Several authors have reported that low concentrations of adenosine increase the sensitivity of rat adipocytes to the antilipolytic action of insulin, and that addition of adenosine deaminase to incubation media may oppose effects attributable to endogenous adenosine released by the cells (see the introduction for references). The sensitivity to the antilipolytic action of insulin was therefore re-examined in incuba-

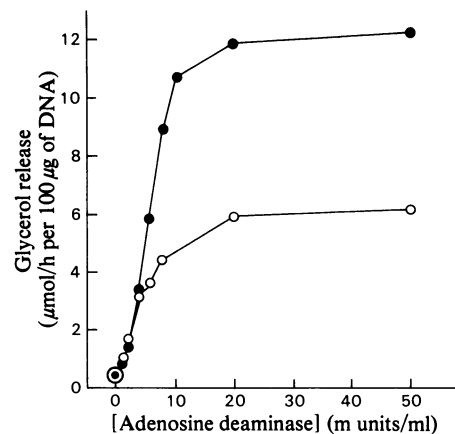


Fig. 3. Effect of adenosine deaminase on basal lipolysis in adipocytes from adrenalectomized and sham-operated rats

Adipocytes from adrenalectomized (○) or sham-operated (●) rats were incubated for 1 h with the indicated concentrations of adenosine deaminase. The results are the means of two independent measurements.

tions containing adenosine deaminase (Fig. 2), which was present at 40–50 munits/ml in all experiments, since this concentration of enzyme gave maximum

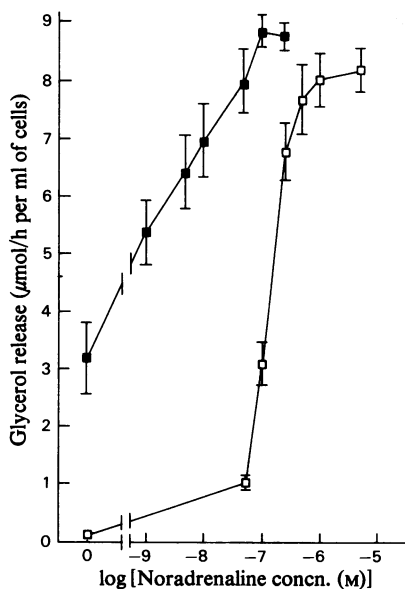


Fig. 4. Lipolytic dose responses to noradrenaline by adipocytes from normal rats in the presence and absence of adenosine deaminase

Adipocytes were incubated for 1 h in the presence (■) or absence (□) of adenosine deaminase (50 munits/ml) and the indicated concentrations of noradrenaline. The results are the means of five independent measurements and the bars indicate S.E.M. The rates of lipolysis are expressed per ml of stock fat-cell suspension, which contained approx. 25 µg of DNA/ml.

stimulation of basal lipolysis (Fig. 3). Adenosine deaminase, besides increasing basal lipolysis (Fig. 3, also see Schwabe & Ebert, 1974; Fain & Wieser, 1975), also increases the sensitivity of adipocyte preparations to noradrenaline (Fig. 4, also see Schwabe & Ebert, 1974; Fain & Wieser, 1975). Also, in the absence of adenosine, insulin is ineffective in opposing the lipolytic action of high concentrations of noradrenaline (Schwabe *et al.*, 1974; Fain & Wieser, 1975). For this reason 0.25 µM-noradrenaline, which was used in the absence of adenosine deaminase (Fig. 1), was too high a concentration to use in the presence of adenosine deaminase. Fig. 4 shows that with cells from normal rats identical rates of lipolysis were achieved with 0.25 µM-noradrenaline (with no adenosine deaminase) or with 8 nM-noradrenaline + adenosine deaminase. Accordingly, 8 nM-noradrenaline was used in the experiment summarized in Fig. 2. Absolute rates of lipolysis were slightly less in cells from adrenalectomized rats under these conditions. Fig. 2 shows that in the presence of adenosine deaminase there was no significant difference in sensitivity to insulin between cells from

adrenalectomized and sham-operated animals. The need to use a different noradrenaline concentration in the experiments shown in Figs. 1 and 2 is obviously unsatisfactory and limits the interpretation of the results. Unfortunately, this cannot be avoided, for reasons discussed above. It is tentatively concluded that adenosine may play some part in the differential insulin sensitivity seen after adrenalectomy.

Effect of adrenalectomy on the response of adipocytes to lipolytic agents

Fig. 5 shows that in the absence of adenosine deaminase rates of noradrenaline-stimulated lipolysis in cells from adrenalectomized rats were approximately 60% of those in cells from sham-operated animals ($P < 0.01$, < 0.01 , < 0.001 , < 0.02 and < 0.01 at 0.1 µM, 1 µM, 10 µM, 0.1 mM and 1 mM respectively for comparison of the adrenalectomized and sham-operated conditions). Between 0.1 µM- and 100 µM-noradrenaline the percentage stimulation above basal lipolysis varied between 1800 and 2600% for cells from sham-operated rats and between 1200 and 2000% for cells from adrenalectomized animals. There was therefore no great change in sensitivity to noradrenaline after adrenalectomy. Exton *et al.* (1972) have shown quite similar decreases in maximum lipolytic rates with noradrenaline in adipocytes from adrenalectomized rats, whereas Skidmore *et al.* (1972), using incubated fat-pieces, found decreases of a larger magnitude. The experiment shown in Fig. 5 covered a wide range of noradrenaline concentrations, but it is noteworthy that biphasic dose responses to noradrenaline reported by Allen *et al.* (1969) were not observed. The reason for this difference is unclear. Fig. 6 shows that the difference in maximum lipolytic response to noradrenaline observed between cells from adrenalectomized and sham-operated rats is abolished in the presence of adenosine deaminase. Also, the maximum rates of lipolysis seen in the presence of adenosine deaminase in the adrenalectomized conditions were similar to those observed in the absence of the enzyme with cells from sham-operated rats (cf. values in Figs. 5 and 6).

Lipolytic dose responses to corticotropin in the absence of adenosine deaminase are shown in Fig. 7(a). After adrenalectomy a decreased sensitivity to low, but not higher, concentrations of this hormone was clearly seen ($P < 0.001$, < 0.001 and < 0.01 at 2.5, 12.5 and 25 ng of corticotropin/ml respectively for comparison of the adrenalectomized and sham-operated conditions). This change in lipolytic response to low concentrations of corticotropin is in accord with the observations made by Exton *et al.* (1972) with isolated adipocytes and by Schotz *et al.* (1959) with incubated fat-pads. Rates of lipolysis in the presence of a high corticotropin concentration

(0.25 $\mu\text{g/ml}$) and adenosine deaminase were similar with both types of cells.

Lipolytic dose responses to glucagon in the absence of adenosine deaminase are shown in Fig. 7(b) and confirm previous findings that the maximum lipolytic response to this hormone is less than that seen with adrenaline, noradrenaline or corticotropin

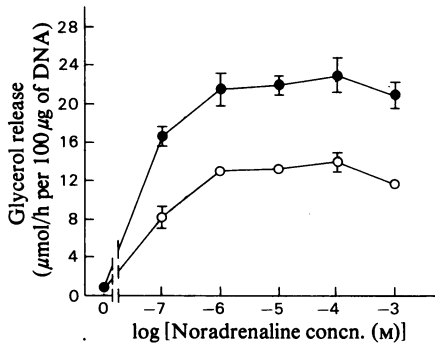


Fig. 5. Lipolytic dose responses to noradrenaline by adipocytes from adrenalectomized and sham-operated rats

Adipocytes from adrenalectomized (○) or sham-operated (●) rats were incubated for 1 h with the indicated concentrations of noradrenaline. The results are the means of four independent measurements and the bars indicate s.e.m. Where no bars are shown, they lie within the area of the symbol.

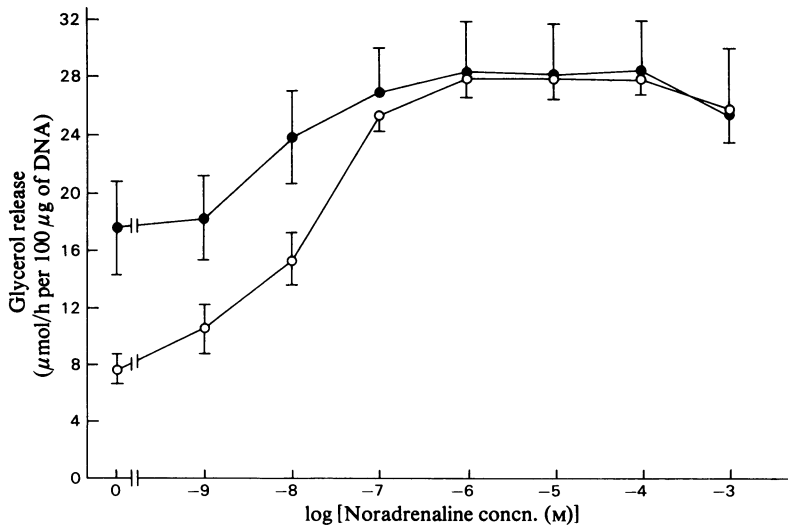


Fig. 6. Lipolytic dose responses to noradrenaline in the presence of adenosine deaminase by adipocytes from adrenalectomized and sham-operated rats

Adipocytes from adrenalectomized (○) or sham-operated (●) rats were incubated for 1 h with adenosine deaminase (50 munits/ml) and the indicated concentrations of noradrenaline. The results are the means of three independent measurements and the bars indicate s.e.m.

(Vaughan, 1960; Hagen, 1961; Blecher *et al.*, 1969). Adipocytes from adrenalectomized rats were clearly extremely insensitive to glucagon compared with cells from sham-operated animals ($P < 0.02$, for all glucagon concentrations from 0.125 to 0.5 $\mu\text{g/ml}$ for comparison of the adrenalectomized and sham-operated conditions). We are unaware of any previous reports on the effects of adrenalectomy on the lipolytic response to this hormone. It is noteworthy that phosphorylase *a* activity (Saitoh & Ui, 1975) and gluconeogenesis (Exton *et al.*, 1972) show remarkable insensitivity to glucagon in perfused livers from starved adrenalectomized rats. In the presence of adenosine deaminase the difference in lipolytic response to glucagon observed after adrenalectomy was abolished. In addition the rate of lipolysis in both types of cells was considerably increased by adenosine deaminase (at 0.25 μg of glucagon/ml, $P < 0.01$ and $P < 0.001$ for sham-operated and adrenalectomized conditions respectively). The lipolytic rates with glucagon + adenosine deaminase were then similar to those seen with noradrenaline, corticotropin or theophylline in the presence of this enzyme (Figs. 6, 7a and 7c). It is pertinent that Fain (1973) has observed that increased 3':5'-cyclic AMP content after glucagon stimulation of rat adipocytes can be very strongly suppressed by low concentrations of adenosine.

In contrast with the findings of Allen & Beck (1972) the lipolytic response to theophylline was not appreciably altered by adrenalectomy (Fig. 7c).

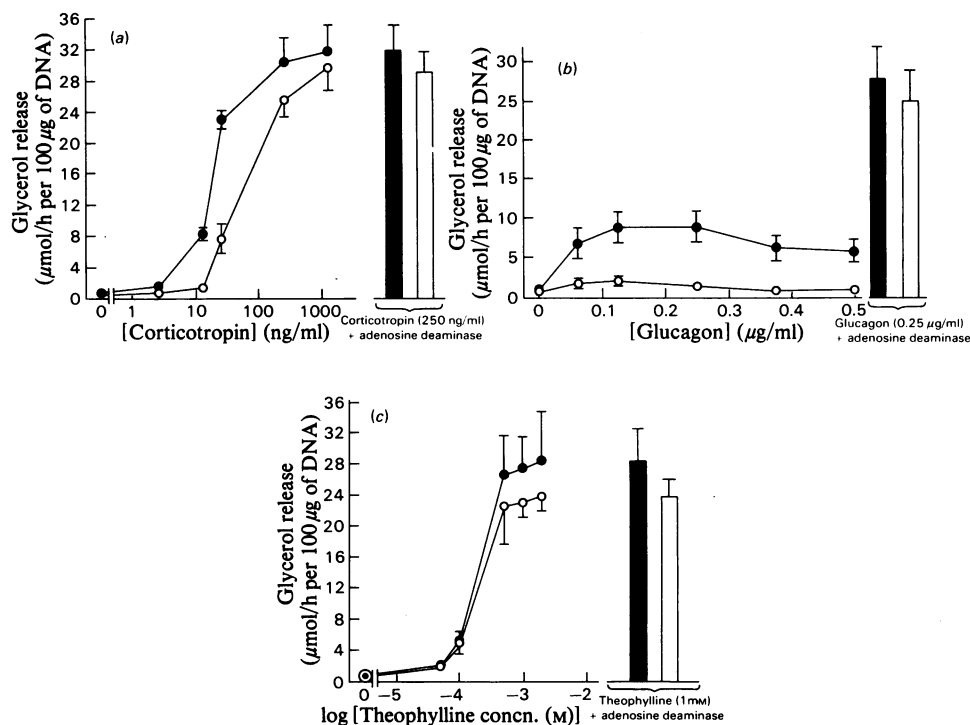


Fig. 7. Lipolytic dose responses to corticotropin, glucagon and theophylline by adipocytes from adrenalectomized and sham-operated rats

Adipocytes from adrenalectomized (open symbols and histograms) or sham-operated rats (closed symbols and histograms) were incubated for 1 h with the indicated concentrations of corticotropin, glucagon or theophylline and, where indicated, adenosine deaminase (45–50 munits/ml). The bars indicate S.E.M. (a) Corticotropin, four independent measurements; (b) glucagon, four independent measurements; (c) theophylline, three independent measurements.

Adenosine deaminase had no effect on lipolysis elicited by high concentrations of theophylline.

Taking the means of all the values measured in these experiments, basal lipolysis in the absence of adenosine deaminase was $1.3 \pm 0.4 \mu\text{mol/h}$ per $100 \mu\text{g}$ of DNA in the sham-operated condition and $0.5 \pm 0.6 \mu\text{mol/h}$ per $100 \mu\text{g}$ of DNA after adrenalectomy (no significant change). This has been reported to be significantly decreased by adrenalectomy in incubated fat-pieces (Skidmore *et al.*, 1972) and in adipocytes (Smith & Saggerson, 1977), or to be essentially unchanged in adipocytes (Exton *et al.*, 1972; Allen & Beck, 1972). These discrepancies may perhaps reflect varied release and accumulation of adenosine in different preparations. In the presence of adenosine deaminase the basal lipolytic rate was $16.5 \pm 3.1 \mu\text{mol/h}$ per $100 \mu\text{g}$ of DNA in cells from sham-operated rats and $8.7 \pm 0.9 \mu\text{mol/h}$ per $100 \mu\text{g}$ of DNA after adrenalectomy ($P < 0.05$ for comparison of the two conditions). In the presence of adenosine deaminase and high concentrations of noradrenaline,

corticotropin, glucagon or theophylline, however, all lipolytic rates were essentially similar (Figs. 6 and 7), suggesting that some component of the 'lipolytic cascade' was then operating at maximal rate and is rate-limiting in both conditions.

General considerations

Although other explanations obviously can be advanced to explain the present findings, it is reasonable to suggest that the changes in hormone response observed after adrenalectomy could result from increased formation or decreased degradation of adenosine by the adipocytes from adrenalectomized rats, increased adenosine release, or even increased sensitivity of the cells to adenosine. The amounts of adenosine released by incubated adipocytes that are able to profoundly alter the hormonal response of the tissue are extremely small (Schwabe *et al.*, 1973). For this reason, our attempts to detect differences in adenosine release between preparations

from adrenalectomized and sham-operated rats have been unsuccessful.

Corbin & Park (1969) and Exton *et al.* (1972) have proposed that the lesion in lipolysis after adrenalectomy results from a decreased sensitivity of the process to 3':5'-cyclic AMP. On this basis our present finding, that adenosine deaminase corrects the lesion in responsiveness to noradrenaline or glucagon, could be simply explained by increased 3':5'-cyclic AMP accumulation, resulting from adenosine removal, overcoming this proposed desensitization to 3':5'-cyclic AMP. Exton *et al.* (1972) supported their proposal by the finding that adrenaline stimulation of glycerol release is impaired in epididymal fat-pads or adipocytes from adrenalectomized rats, whereas 3':5'-cyclic AMP accumulation in response to adrenaline is increased in fat-pads from these animals. However, two studies with isolated adipocytes (Allen & Beck, 1972; Schönhöfer *et al.*, 1972) have shown that 3':5'-cyclic AMP accumulation in response to noradrenaline, adrenaline or corticotropin is decreased after adrenalectomy. On the basis of this and other findings, Schönhöfer *et al.* (1972) and Skidmore *et al.* (1972) have concluded that the lesion in responsiveness to lipolytic hormones after adrenalectomy lies in the 'information process' or 'coupling' between hormonal receptors and adenylate cyclase. It is noteworthy that Birnbaumer (1973) has discussed the involvement of purine derivatives including adenosine in the modulation of such coupling processes. The inconsistency of the findings of Exton *et al.* (1972) with those of Allen & Beck (1972) and Schönhöfer *et al.* (1972) could be explained by the fact that Exton *et al.* (1972) made 3':5'-cyclic AMP measurements in fat-pads that must contain other cell types besides adipocytes and also nerve endings. It is pertinent that Sattin & Rall (1970) observed that adenosine potentiates noradrenaline stimulation of 3':5'-cyclic AMP accumulation in nervous tissue, which is directly opposite to the effect of this nucleoside in adipocytes (Fain *et al.*, 1972; Schwabe *et al.*, 1973; Fain & Wieser, 1975). Our finding that the extent and nature of the lesion in responsiveness to lipolytic agents after adrenalectomy differs with the type of hormonal stimulus (cf. Figs. 5, 7a and 7b) supports the contention of Schönhöfer *et al.* (1972) that this lesion must be 'early' rather than 'late' in the chain of events leading to stimulation of lipolysis. This is also supported by the finding that theophylline (Fig. 7c) appears to bypass this lesion, possibly by acting solely as a cyclic nucleotide phosphodiesterase inhibitor, or possibly by additionally acting as a methylxanthine antagonist to adenosine as in other systems (Nicholls & Walaszek, 1963; De Gubareff & Sleator, 1965; Guthrie & Nayler, 1967; Afonso & O'Brien, 1970). It is noteworthy that adrenalectomy decreases Ca²⁺ exchange in adipose tissue (Werner

et al., 1972), and that adenosine has a similar action in cardiac preparations (Grossman & Furchgott, 1964; Guthrie & Nayler, 1967). Also the effects of adrenalectomy on cardiac contractility and Ca²⁺ exchange (Gerlach & van Zwieten, 1969; Rovetto & Lefer, 1970) are similar to the actions of adenosine. Finally, *in vivo* adenosine administration completely opposes the effect of cortisol treatment, which promotes hepatic glycogen accumulation in adrenalectomized rats (De Sánchez *et al.*, 1971).

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