Rat fat-cells have three types of adenosine receptors (R_a, R_i and P)

Differential effects of pertussis toxin

J. Adolfo GARCÍA-SÁINZ and M. Luz TORNER
Departamento de Bioenergética, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Apartado Postal 70-600, 04510 México D.F., Mexico

Activation of rat adipocyte R_i adenosine receptors by phenylisopropyladenosine (PIA) decreased cyclic AMP and lipolysis; this effect was blocked in cells from pertussis-toxin-treated rats. In contrast, the ability of 2′,5′-dideoxyadenosine to decrease cyclic AMP was not affected by pertussis-toxin treatment. Addition of adenosine deaminase to the medium in which adipocytes from control animals were incubated resulted in activation of lipolysis. Interestingly, adipocytes from toxin-treated rats (which had an already increased basal lipolysis) responded in an opposite fashion to the addition of adenosine deaminase, i.e. the enzyme decreased lipolysis, which suggested that adenosine might be increasing lipolysis in these cells. Studies with the selective agonists *N*-ethylcarboxamidoadenosine (NECA) and PIA indicated that adenosine increases lipolysis and cyclic AMP accumulation in these cells and that these actions are mediated through R_a adenosine receptors. Adenosine-mediated accumulation of cyclic AMP was also observed in cells preincubated with pertussis toxin (2 μg/ml) for 3 h. In these studies NECA was also more effective than PIA. Our results indicate that there are three types of adenosine receptors in fat-cells, whose actions are affected differently by pertussis toxin, i.e. R_i-mediated actions are abolished, R_a-mediated actions are revealed and P-mediated actions are not affected.

INTRODUCTION

Dole (1961) was the first to report that adenosine decreases the lipolytic effect of adrenaline in rat fat-pads. Later, many other groups confirmed his results and demonstrated that not only adenosine but also other related compounds (such as ATP, ADP, AMP, NAD^+, $NADP^+$ etc.) inhibited hormone-induced lipolysis in adipose tissue.

A second significant advance in our understanding of adenosine action of fat-cells was the finding by Schwabe and co-workers that this nucleoside is continuously released by adipocytes into the incubation medium in amounts which inhibit cyclic AMP accumulation and lipolysis (Schwabe *et al.*, 1973, 1975; Schwabe & Ebert, 1974). This has been confirmed and extended by many other groups (Fain *et al.*, 1972; Turpin *et al.*, 1977; Fain, 1979; Fredholm & Hjemdahl, 1979).

Another important achievement was the study of the pharmacology of adenosine action by Londos and co-workers (Londos & Wolff, 1977; Londos *et al.*, 1978, 1980). They observed, using different adenosine analogues, that there are three types of adenosine receptors: (*a*) P-sites, with strict structural specificity with respect to the purine moiety of the molecule (these sites mediate inhibition of adenylate cyclase and are putatively located on the intracellular face of the plasma membrane); and (*b*) two subclasses of external adenosine receptors, which have structural specificity for the ribose moiety of the molecule (thus termed 'R'-sites); one of these subclasses of receptors mediates activation of adenylate cyclase (R_a), whereas the other mediates inhibition (R_i). These receptor subclasses have been discriminated by using selective adenosine agonists (Londos & Wolff, 1977; Londos *et al.*, 1978, 1980).

Current ideas indicate that only two of these three types of adenosine receptors (P and R_i sites), both of which mediate inhibition of adenylate cyclase, are present in fat-cells (Fain, 1973; Fain & Malbon, 1979). We have performed a systematic study of the actions of pertussis toxin on the effects of adenosine in fat-cells and observed that pertussis toxin blocks the inhibition of cyclic AMP accumulation and lipolysis produced by PIA, a selective R_i agonist (García-Sáinz, 1981; Martínez-Olmedo & García-Sáinz, 1983, 1984). The inhibition of cyclic AMP accumulation in fat-cells produced by 2′,5′-dideoxyadenosine, a selective agonist for P-sites, is not blocked by pertussis toxin (Martínez-Olmedo & García-Sáinz, 1983). To our surprise, we observed some effects of adenosine on lipolysis and cyclic AMP accumulation that could not be explained on the basis of our previous findings; these results are presented here, and clearly indicate that fat-cells contain the three types of adenosine receptors and that their actions are affected differently by pertussis toxin.

MATERIALS AND METHODS

PIA was obtained from Boehringer Mannheim, 2′,5′-dideoxyadenosine from P-L Biochemicals, and NECA was generously given by Byk-Gulden Pharmazeutika (Konstanz, West Germany). Adenosine deaminase and prostaglandin E_2 were from Sigma Chemical Co. Collagenase and bovine serum albumin (fraction V) were obtained from Worthington and Armour respectively. Cyclic [^{3}H]AMP was from New England Nuclear. Pertussis toxin was purified from pertussis-vaccine concentrates (generously provided by the National Institutes of Hygiene of Mexico) as previously described

Abbreviations used: PIA, phenylisopropyladenosine; NECA, 5′-*N*-ethylcarboxamidoadenosine.

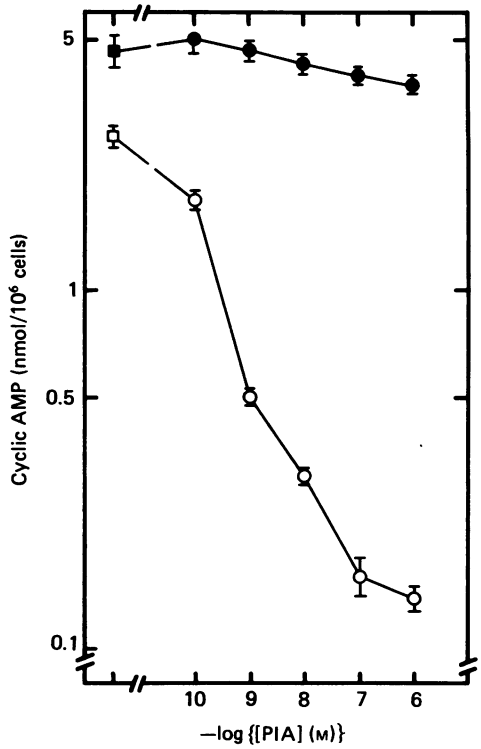

Fig. 1. Effect of PIA on the cyclic AMP accumulation produced by isoprenaline in fat-cells from control (○) and pertussis-toxin-treated (●) rats

Adipocytes were incubated for 10 min in the presence of 1 μg of adenosine deaminase/ml, 0.1 μM-isoprenaline and different concentrations of PIA. Basal cyclic AMP concentrations in the absence of isoprenaline were 0.15±0.01 and 0.13±0.01 nmol/10⁶ cells in cells from control and pertussis-toxin-treated rats respectively. Results are means±S.E.M. for eight determinations using different preparations in each case.

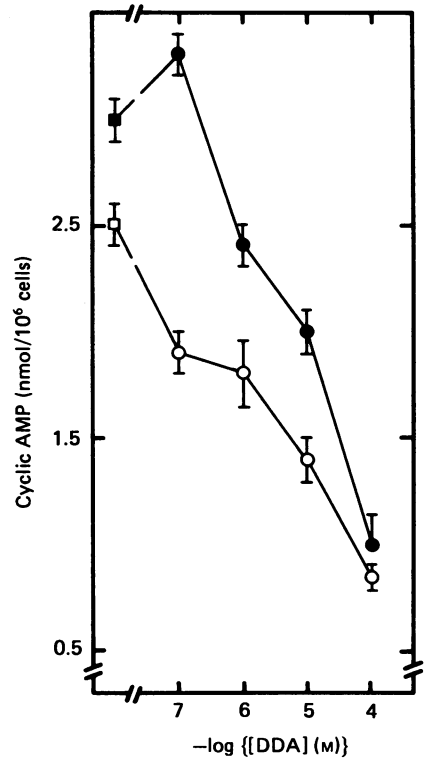

Fig. 2. Effect of dideoxyadenosine (DDA) on the cyclic AMP accumulation produced by isoprenaline in fat-cells from control (○) and pertussis-toxin-treated (●) rats

Adipocytes were incubated for 10 min in the presence of 1 μg of adenosine deaminase/ml, 0.1 μM-isoprenaline and different concentrations of DDA. For other details, see Fig. 1.

(Martínez-Olmedo & García-Sáinz, 1983, 1984) or by the method of Sekura *et al.* (1983). Both methods resulted in preparations with identical purity and potency, but the latter was simpler and faster.

Male Wistar rats fed *ad libitum* were used. Pertussis toxin (10 μg/100 g) was administered intraperitoneally to the animals 3–6 days before the experiment was performed. Adipocytes were obtained by collagenase digestion of epididymal and perirenal fat-pads (Rodbell, 1964). The pads were digested and the cells incubated in Krebs–Ringer bicarbonate buffer (Tolbert *et al.*, 1980) supplemented with 3% (w/v) bovine serum albumin, pH 7.4 at 37 °C, under an atmosphere of O_2/CO_2 (19:1). Incubations were in 1 ml of buffer at a cell density of approx. 2×10^5 cells/ml. The incubations were carried out for 10 min to determine cyclic AMP accumulation and for 60 min to measure non-esterified fatty acid release, which was considered as an index of lipolysis, since under the conditions described (absence of glucose) no re-esterification occurred (Vaughan, 1962). Cyclic AMP accumulation was determined by the method of Gilman (1970), as modified by Brown *et al.* (1971). Non-esterified fatty acids were quantified by the method of Novak (1965).

In the experiments presented in Fig. 5, isolated adipocytes were incubated for 3 h in the absence or presence of pertussis toxin. After this preincubation, the cells were washed and incubated for 10 min with the agents indicated to determine cyclic AMP accumulation.

RESULTS

Stimulation of fat-cells from control animals with 0.1 μM-isoprenaline produced a marked accumulation of cyclic AMP (Fig. 1). Such stimulation was even bigger in cells from pertussis-toxin-treated rats ($P < 0.001$; Fig. 1), confirming previous findings that pertussis toxin magnifies the action of agents that stimulate adenylate cyclase (Katada & Ui, 1982; Katada *et al.*, 1982; Martínez-Olmedo & García-Sáinz, 1983, 1984; García-Sáinz *et al.*, 1984). PIA inhibited in a dose-dependent fashion the accumulation of cyclic AMP produced by 0.1 μM-isoprenaline (Fig. 1). In contrast, nearly no decrease in cyclic AMP was produced by PIA in cells from pertussis-toxin-treated animals.

2′,5′-Dideoxyadenosine significantly decreased the accumulation of cyclic AMP produced by β-adrenergic activation in adipocytes from both control and pertussis-toxin-treated animals (Fig. 2). Both the shape of the curve and the magnitude of the effect indicated that the ability of dideoxyadenosine to inhibit adenylate cyclase is not affected by pertussis toxin (Fig. 2).

These data essentially confirm our previous findings

Table 1. Effect of adenosine deaminase, PIA and prostaglandin E_2 on lipolysis in fat-cells from control and pertussis-toxin-treated rats

Adipocytes were incubated for 60 min under the conditions described in the Materials and methods section. Results are expressed as the means ± S.E.M. for at least eight determinations with different cell preparations. Abbreviation: ND, not determined. [a] $P < 0.001$ compared with basal controls; [b] $P < 0.001$ compared with its respective basal group; [c] $P < 0.001$ compared with its respective adenosine deaminase-treated group.

	Non-esterified fatty acids (μequiv./10^6 cells)	
	Control	Pertussis-toxin-treated
Basal	0.80 ± 0.05	10.30 ± 0.50[a]
Adenosine deaminase (1 μg/ml)	2.60 ± 0.30[b]	6.10 ± 0.40[b]
Adenosine deaminase + PIA (1 μM)	0.95 ± 0.10[c]	9.70 ± 0.30[c]
Adenosine deaminase + prostaglandin E_2 (10 μM)	ND	6.50 ± 0.50

(Garcĵa-Sáinz, 1981; Martínez-Olmedo & García-Sáinz, 1983, 1984). However, when lipolysis was studied, some surprising results were observed, which are listed in Table 1. As expected, basal lipolysis was increased in cells from pertussis-toxin-treated rats as compared with the controls (Table 1), putatively by blocking adenosine action through R_i adenosine receptors. Removal of endogenous adenosine by addition of adenosine deaminase to the medium resulted in increased lipolysis in cells from control animals (Table 1), but to our surprise adenosine deaminase significantly decreased basal lipolysis in cells from toxin-treated rats (Table 1). This result was particularly puzzling and was confirmed many times with different concentrations of adenosine deaminase and different lots of the enzyme. We finally accepted the evidence; removal of endogenous adenosine resulted in decreased basal lipolysis in these cells. Furthermore, addition of PIA (which normally decreases the stimulation of lipolysis produced by adenosine deaminase; Table 1) stimulated lipolysis in cells from toxin-treated rats (Table 1).

Two possibilities were considered to explain these findings: firstly, that activation of R_i adenosine receptors in cells from toxin-treated rats could result in stimulation of adenylate cyclase activity, and secondly, the presence of R_a adenosine receptors in fat-cells.

To evaluate the first possibility, the action of other agents that inhibit adenylate cyclase in fat-cells was studied. Prostaglandin E_2 is such an agent. It was absolutely unable to mimic the lipolytic effect of PIA (Table 1).

To evaluate the possible existence of R_a adenosine

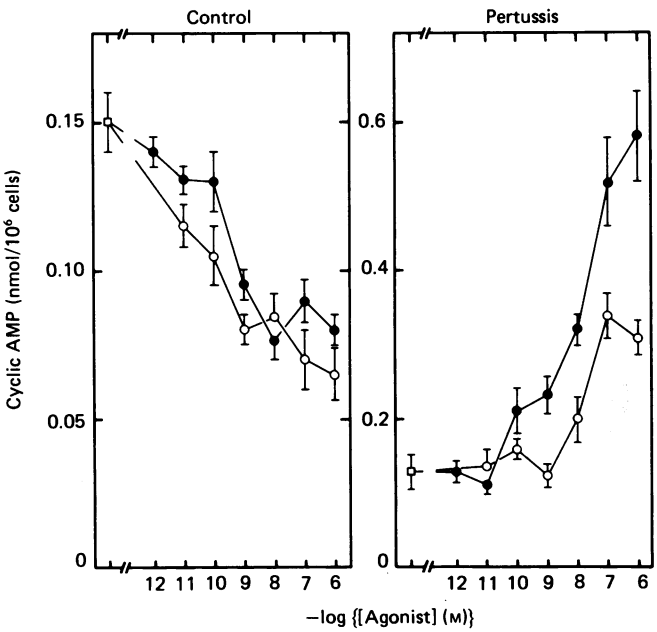

Fig. 3. Effect of adenosine agonists on cyclic AMP accumulation in fat-cells from control and pertussis-toxin-treated rats

Adipocytes were incubated for 10 min in the presence of 1 μg of adenosine deaminase/ml and different concentrations of PIA (○) or NECA (●). For other details, see Fig. 1.

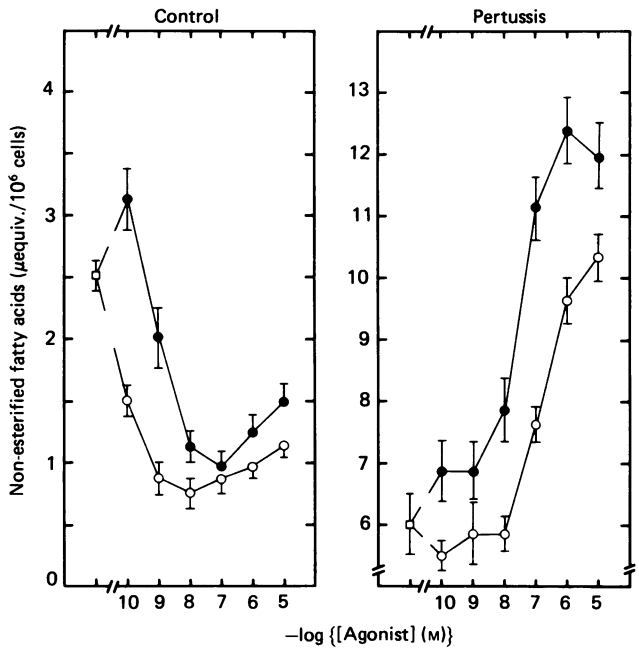

Fig. 4. Effect of adenosine agonists on non-esterified fatty acid release in fat-cells from control and pertussis-toxin-treated rats

Adipocytes were incubated for 60 min in the presence of 1 μg of adenosine deaminase/ml and different concentrations of PIA (○) or NECA (●). Basal values for non-esterified fatty acid release are shown by □ and in Table 1. For other details see Fig. 1.

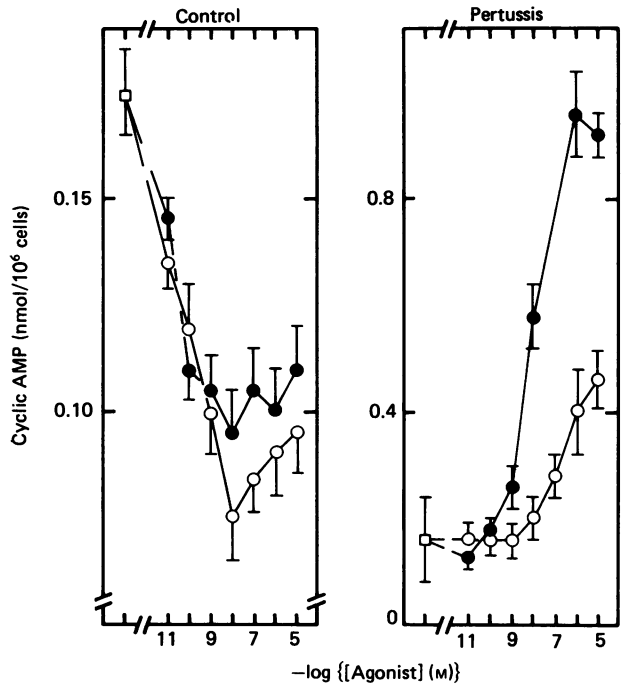

Fig. 5. Effect of adenosine agonists on cyclic AMP accumulation in fat-cells incubated in the absence or presence of pertussis toxin

Adipocytes were incubated in the absence or presence of pertussis toxin (2 μg/ml) for 3 h. After this period, the cells were washed and incubated for 10 min in the presence of 1 μg of adenosine deaminase/ml and different concentrations of PIA (○) or NECA (●). For other details, see Fig. 1.

receptors in fat-cells, experiments with the selective R_a agonist NECA were performed. In cells from control animals, both PIA and NECA were able to decrease basal cyclic AMP concentrations (Fig. 3). As expected, PIA was more potent than NECA for this effect. In cells from toxin-treated rats, both adenosine agonists produced dose-dependent increases in cyclic AMP (Fig. 3). Interestingly, NECA and PIA had similar potencies for this effect, but NECA was significantly more effective than PIA, i.e. NECA increased cyclic AMP 4.7-fold, whereas PIA only increased it 2.7-fold.

Similar contrasting results were observed when lipolysis was studied (Fig. 4): both adenosine agonists inhibited lipolysis in cells from control animals, whereas they stimulated lipolysis in cells from toxin-treated rats (Fig. 4). PIA was more potent than NECA for inhibiting lipolysis, whereas NECA was more potent and effective than PIA for activating lipolysis (Fig. 4). All these results clearly indicate the existence of R_a adenosine receptors in fat-cells from toxin-treated rats.

The effect was reproduced by incubating isolated adipocytes with the toxin (2 μg/ml for 3 h). Under these conditions the cells increased their cyclic AMP in response to adenosine analogues; NECA was significantly more effective than PIA (6-fold and 2.9-fold increase respectively) (Fig. 5). It was observed that lipolysis was maximally stimulated by the toxin, and no further stimulation was produced.

DISCUSSION

This paper is the first to show the presence of R_a adenosine receptors in fat-cells. The data are consistent with the present model for the actions of adenosine on adenylate cyclase activity, i.e. R_a receptors are linked to stimulation of adenylate cyclase and R_i and P receptors are linked to inhibition. Pertussis toxin affects differently the action of each type of receptor, i.e. R_a-mediated actions are revealed, R_i-mediated actions are inhibited and P-mediated actions are not affected. Pertussis toxin seems to exert its actions by ADP-ribosylation of the inhibitory guanine-nucleotide-binding regulatory protein (N_i) (Katada & Ui, 1982; Malbon *et al.*, 1984). This covalent modification of N_i blocks the action of receptors that are coupled to adenylate cyclase through it, such as the R_i adenosine receptors. The P adenosine receptor seems to interact directly with the cyclase, by-passing N_i; thus its action is not affected by pertussis toxin. The enhanced action of receptors coupled in an activatory fashion to adenylate cyclase, such as the R_a adenosine receptor, is probably the result of an altered interplay of the subunits of the guanine-nucleotide regulatory proteins (N_i and N_s), induced by the covalent modification of N_i by pertussis toxin (Katada *et al.*, 1984; Rich *et al.*, 1984).

The action of adenosine through R_a adenosine receptors is not evident under basal conditions, probably because of an enormous predominance of R_i adenosine receptors in the cells. However, blockage of N_i by pertussis toxin revealed the presence of R_a adenosine receptors. It should be mentioned, however, that the accumulation of cyclic AMP produced by activation of R_a adenosine receptors is rather small compared with that produced by β-adrenergic agents. This suggests that either the number of this type of receptors is relatively small or that their coupling is not very effective.

We thank Dr. J. Garza and Dr. H. Villalva for the generous donation of pertussis-vaccine concentrates, Dr. T. Dunwiddie for the gift of some substances, and Mrs. Guadalupe Ramírez for carefully typing the manuscript. This research was partially supported by a Grant from Fondo de Estudios e Investigaciones 'Ricardo J. Zevada'. J.A.G.-S. is a 1985–1986 Guggenheim Fellow.

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Received 3 June 1985/22 July 1985; accepted 26 July 1985