Metabolism
Clinical and Experimental

VOL 46, NO 6 JUNE 1997

Acute Effects of Progesterone on Glucose Metabolism in Rat Adipocytes: Are They Modulated by Endogenous Adenosine?

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Progesterone rapidly inhibits glucose oxidation of isolated rat adipocytes. Because this inhibition is triggered by endogenous adenosine, the present study was designed to examine the effect of the steroid on cyclic adenosine monophosphate (cAMP) accumulation, its relation to lipolysis, and the possible participation of adenosine. The results strongly indicate that physiological concentrations of progesterone increase the release of adenosine by isolated adipocytes, with maximal release at the end of a 20-minute incubation. Progesterone decreased both cAMP levels and lipolysis in quiescent adipocytes or in adipocytes stimulated by isoproterenol. The increase of endogenous adenosine may explain the decline of cAMP and glycerol levels observed with progesterone. The effects of the steroid on lipolysis disappeared when adenosine was hydrolyzed by adenosine deaminase (ADA). On the other hand, in the absence of endogenous adenosine, the effect of progesterone on the cAMP level was decreased only in isoproterenol-stimulated cells. The inhibitory effects of progesterone on cAMP and glycerol production seem not to be related directly to the adenosine A_1 receptor, for selective A_1 receptor antagonists (8-cyclopentyl-1,3-dipropylxanthine [DPCPX] and CP 68,247) did not counteract these effects. However, mechanisms mediated by guanyl nucleotide binding proteins cannot be excluded. The decrease of cAMP and of lipolysis may be related to a stimulation of phosphodiesterases (PDEs). When PDEs I (Ca^{2+} – calmodulin-regulated PDE family) were blocked by a selective inhibitor (CP 41,757), the progesterone inhibitory effect persisted, suggesting that PDEs I are not regulated by the steroid. On the other hand, the progesterone effect on cAMP accumulation but not on lipolysis disappeared in the presence of a selective inhibitor of the PDE IV family (cAMP-dependent–specific family), Ro 20-1724. When the specific inhibitor of PDE I or PDE IV was combined with ADA, the progesterone effect on cAMP disappeared. Taken together, these results suggest that the progesterone inhibitory action on cAMP levels was not mediated through A_1 receptors or through activation of PDE I, but may be related to PDE IV activities. The progesterone effect on lipolysis seemed not to be directly related to changes in cAMP levels; an effect on PDE III activities in relation with the increase of adenosine release cannot be excluded.
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PROGESTERONE administered long-term to animals[1-3] or added in vitro to isolated adipose cells or to muscles[4-6] decreases glucose metabolism. With isolated adipose cells, inhibition was seen after only 20 minutes of incubation with the steroid[7] and was accompanied by enhanced protein and decreased fatty acid synthesis.[8] Actinomycin D, which inhibits protein synthesis by its action on nuclear RNA synthesis,[9] had no effect either on this increased protein synthesis[10] or on the inhibition of glucose oxidation.[11] Although the mechanism(s) of the actions of progesterone on glucose metabolism is poorly understood, it seems likely to be genomic and nongenomic.

Other acute actions of steroids have been noted in different cell types,[12,13] some of them mediated by cyclic adenosine monophosphate (cAMP): reinitiation of meiosis in *Xenopus* oocytes,[14] regulation of activities of phosphodiesterases (PDEs) in rat[15] or in human uterine tissues,[16] or modulation of lipolysis in adipose cells.[17]

These observations led us to look for possible effects of progesterone on cAMP levels in isolated adipocytes and on consequent changes in lipolysis due to activation of triglyceride lipase via cAMP-dependent protein kinase.[18,19] We have shown recently that glucose metabolism was increased at low progesterone concentrations (10^{-10} to 10^{-8} mol/L) and inhibited at higher levels (10^{-7} to 10^{-4} mol/L). Adenosine released from incubated adipose cells appears to play a physiologic role in fat cell metabolism[20] by inhibiting hormone-stimulated cAMP accumulation, glycerol release,[21,22] and glucose oxidation.[23] When endogenous adenosine was hydrolyzed by added adeno-

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Submitted March 18, 1995; accepted January 3, 1997.

Supported by grants from the Fondation pour la Recherche Médicale Française and the Conseil Régional d'Aquitaine.

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0026-0495/97/4606-0001$03.00/0

sine deaminase, the steroid effect was only inhibitory.[11] Since these results suggest a regulatory role of adenosine in the effect of progesterone, the present studies were designed to examine whether the actions of the steroid on cAMP levels[24] and on lipolysis[6,25] are also mediated by adenosine. In this regard, our cell incubations were performed in the absence or presence of adenosine deaminase ([ADA] which converts adenosine to inosine), which stimulates cAMP accumulation and lipolysis.[26] The effect of progesterone after stimulation of cAMP production and lipolysis by isoproterenol was also determined. Further, the influence of the steroid on the release of adenosine into the incubation medium was measured. Finally, with the use of specific antagonists and inhibitors,[27-29] the locus of action of the steroid, mediated by the nucleoside, was determined to be on adenosine A_1 receptors or on the activities of PDEs.

MATERIALS AND METHODS

Materials

Crystalline progesterone (Codex BP 68 NF JP) was generously provided by Roussel-Uclaf (Romainville, France). Collagenase (CLS, type I, 127 U/mg) was obtained from Worthington Biochemicals (Freehold, NJ). Bovine serum albumin, dimethylsulfoxide (DMSO), and 3-isobutyl-1-methylxanthine (IBMX) were from Sigma France (La Verpilliére, France). R (−)N^6-(2-phenylisopropyl) adenosine (R-PIA), R (−)-isoproterenol(+)-bitartrate salt, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), and erythro-9-(2-hydroxy-3 nonyl) adenosine (EHNA) were from RBI (Natick, MA). ADA from calf intestine (200 U/mg) and materials used in the glycerol assay were purchased from Boehringer Mannheim (Meylan, France). Adenosine, the other nucleosides, luminol, and materials for determination of adenosine release were also from Boehringer Mannheim. The cAMP radioimmunoassay kit (reference no. 1117) was obtained from Immunotech (Marseille, France). All other chemicals were purchased from Merck-Clévenot (Nogent-sur-Marne, France).

Ro 20-1724 (Imidazolidinone) was a generous gift from Hoffmann La Roche (Basel, Switzerland), and CP 68,247 and CP 41,757 were gifts from Pfizer (Groton, CT).

Animals

Female Wistar rats were maintained in a temperature (22°C)- and light-controlled room (12-hour light/dark cycle) with free access to laboratory chow (UAR, Epinay-sur-Orge, France) and water. Parametrial and periovarian fat pads were collected from rats weighing 160 to 190 g (46 to 52 weeks). The procedures used were approved by the institutional committee (no. 5223, Ministère de l'Agriculture, France).

Preparation of Isolated Adipocytes

Adipocytes were isolated according to the method of Rodbell[30] with slight modifications.[5] Fat cell viability was assessed by the Trypan blue exclusion test. Cells were counted in a hemocytometer.[5]

Preparation of Solutions

Progesterone first dissolved in absolute ethanol was added to the incubation medium at concentrations of 10^{-10} to 10^{-3} mol/L. The same volume of ethanol was added to the corresponding control vials. Final concentrations of alcohol in the incubation media were always less than 0.1%, and this had no discernible effect on the experimental results.

All inhibitors were made as 20-mmol/L stocks in DMSO[31] and were diluted to the final indicated concentration with Krebs-Ringer bicarbonate-albumin-glucose medium. At the concentrations used, DMSO (added to the control flasks) had no effect on cAMP or on glycerol release.

Fat Cell Incubation

Cell concentration was adjusted to 50,000 ± 5,000/mL. Isolated adipocytes were incubated for 20 minutes (37°C, 100 cycles/min) in Krebs-Ringer bicarbonate buffer containing 2% albumin and 5.6 mmol/L glucose.[5] Drugs were added at time zero as indicated in the figures. At the end of incubation, reactions were stopped by addition of ice-cold trichloroacetic acid (final concentration, 5%).

Extraction of cAMP and Glycerol

cAMP and glycerol were extracted according to the method used by Häring et al[32] with slight modifications. Cells were disrupted with a sonicator (Vibra-Cell; Sonics and Material, Danbury, CT). Centrifugation of the homogenate (15 minutes at 2,000 × *g* and 4°C) separated it into three phases. The upper lipid phase and the bottom phase that contains membranes, precipitated proteins, etc., were discarded. The intermediate phase containing cAMP and glycerol was washed three times with diethylether. After elimination of ether, the samples were frozen at −20°C until assay.

cAMP Determination

cAMP was determined by a radioimmunoassay from Immunotech based on the competitive-binding principle using a monoclonal cAMP antibody. The sensitivity of the assay was 0.2 nmol/L, and the interassay reproducibility was 12% and intraassay 8%. Specificities of the antibody for cAMP, cyclic guanosine monophosphate, 5′AMP, and adenosine triphosphate were respectively 100%, 0.4%, 0.7%, and 0.7%. Specificity for adenosine was not determined. Results are given as picomoles of cAMP per 10^6 cells per incubation time.

Glycerol Determination

An enzymatic procedure derived from the method used by Laurell and Tibbling[33] was used. Results are given as nanomoles of glycerol per 10^6 cells per incubation time.

Adenosine Determination

Cells were incubated by shaking (100 cycles/min at 37°C) for 20 minutes in Krebs-Ringer buffer–albumin containing 5.6 mmol/L glucose and in the presence of 10 μmol/L EHNA, an inhibitor of ADA. Incubation was terminated by immersing vials in a boiling water bath for 1 minute. The contents of the incubation vials were disrupted by sonication and centrifuged for 30 minutes (2,000 × *g* at 4°C), the supernatant fat cake was discarded, and the remaining phase was kept at −20°C until assay. Adenosine was determined by the chemiluminescence method of Kather et al.[34] Results were read against a standard curve constructed with pure adenosine (15.6 to 250 nmol/L) and are given as picomoles of adenosine released per 10^6 cells per incubation time.

Statistical Analysis

Experimental groups presented in Figs 1, 3, 4, and 5 were analyzed at the same time, with adipocytes from each rat represented in each treatment and at each dose of the drugs.

Results are expressed as the mean ± SEM, with the number of independently performed experiments indicated in each figure. Statistical comparisons between different drug treatments were made by the least-squares regression analysis (95% confidence level) using the Graphpad (ISI Software, Philadelphia, PA) program. Because the

different drugs stimulated cAMP and glycerol levels differently, results are expressed as the percentage of each corresponding basal activity.

Unpaired Student's t test was used to show the effect of drugs and progesterone versus basal levels.

RESULTS

Time Course of Glycerol and cAMP Release

The rate of lipolysis in quiescent adipocytes, monitored as basal glycerol release, was relatively constant, increasing from 48.5 ± 2.47 nmol/10^6 cells at time zero to 77.6 ± 9.9 nmol/10^6 cells (n = 7, $P < .01$) at 10 minutes and 92.1 ± 2.1 (n = 3, $P < .05$) at 20 minutes.

At time zero, cAMP concentration was 18.5 ± 2.0 pmol/10^6 cells (n = 11). After stimulation of the cells by glucagon, cAMP peaked at 5 minutes (35.92 ± 7.59 pmol/10^6 cells, n = 8, $P < .01$) and did not change significantly over the next 20 minutes (25.93 ± 2.82 pmol/10^6 cells, n = 4).

An incubation time of 20 minutes was chosen for further studies, since it combined a significant increase in cAMP and glycerol levels with the maximal release of adenosine.

Effect of Progesterone on Adenosine Release

Progesterone (10^{-10} to 10^{-6} mol/L) induced an increased release ($P < .05$ to .01) of adenosine from adipocytes. Pharmacological concentrations of progesterone (10^{-5} to 10^{-3} mol/L) were without significant effect on adenosine levels (Fig 1).

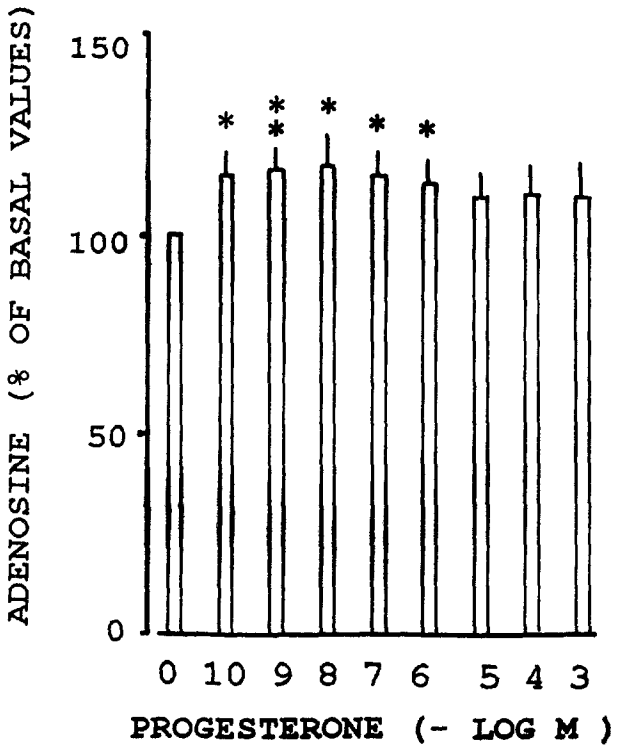

Fig 1. Influence of progesterone on adenosine release by adipocytes in incubation. Adenosine release in the absence of the steroid was 1.67 ± 0.31 nmol/10^6 cells per 20-minute incubation. Each point represents the mean ± SEM of 12 isolated experiments calculated as a percentage of each corresponding control value (absence of progesterone). Effect of progesterone: *$P < .05$, **$P < .01$.

Effect of Progesterone on cAMP and Glycerol Levels

Progesterone (10^{-10} to 10^{-5} mol/L) added to the incubation medium of intact adipocytes decreased cAMP levels and glycerol production. At pharmacological concentrations (10^{-5} and 10^{-4} mol/L), this effect was attenuated (Fig 2).

Action of an Adenosine Analog

R-PIA, a highly potent inhibitor of cAMP formation and lipolysis in the adipose cell, decreases both cAMP and glycerol levels in the presence of ADA: cAMP, 8.89 ± 1.46 pmol/10^6 cells (PIA 10^{-8} mol/L) to 6.53 ± 1.49 (PIA 10^{-7} mol/L) (n = 3) versus 27.9 ± 2.9 (basal value, n = 11, $P < .01$); glycerol, 105.2 ± 7.54 nmol/10^6 cells (PIA 10^{-8} mol/L) to 111.6 ± 10.3 (PIA 10^{-7} mol/L) (n = 4) versus 236 ± 37 (basal value, n = 11, $P < .01$). Progesterone (10^{-9}, 10^{-7}, and 10^{-4} mol/L) produced no further decrease of this effect.

Effect of Progesterone on Different Modes of Lipolytic Stimulation

Glycerol and cAMP release were stimulated by adenosine and by a supramaximal concentration of the β-adrenergic agonist, isoproterenol.

ADA (500 mU/mL) elicited an increase of 129% in the lipolytic response above the basal value (236 ± 37 nmol glycerol/10^6 cells v 103 ± 15, n = 11, $P < .05$), whereas isoproterenol (10^{-6} mol/L) increased it by 1,023% (1,103 ± 25 nmol glycerol/10^6 cells v 98.14 ± 5.53, n = 6, $P < .001$). The combination of isoproterenol with ADA had no further effect on glycerol production (928 ± 64 nmol/10^6 cells).

Further, ADA increased cAMP accumulation by 51% (27.9 ± 2.9 pmol/10^6 cells v 18.5 ± 2.0, n = 11, $P < .05$), whereas isoproterenol increased cAMP levels by 3,860% (684 ± 39 pmol/10^6 cells v 17.27 ± 1.22, n = 6, $P < .001$). In the combined presence of ADA and isoproterenol, cAMP accumulation was further enhanced by 105% (1,403 ± 60 pmol/10^6 cells).

ADA did not abolish the inhibitory effect of progesterone on cAMP accumulation (least-squares regression analysis), except for 10^{-9} and 10^{-8} mol/L progesterone (Student's t test). On the contrary, ADA abolished this effect on glycerol accumulation (least-squares regression analysis) (Fig 2). After isoproterenol stimulation, the inhibitory effect of progesterone on cAMP and glycerol remained (results not shown). However, when ADA was combined with isoproterenol, the inhibitory effect of progesterone both on glycerol and on cAMP levels completely disappeared (results not shown).

Effect of Progesterone on cAMP and Glycerol Levels in the Presence of IBMX

The effect of IBMX on cAMP and glycerol levels was studied in the absence and presence of progesterone (Fig 3). In the presence of 10^{-3} mol/L IBMX, basal levels of cAMP increased 1,700% (333 ± 35 pmol/10^6 cells, n = 7, v 18.5 ± 2.0, n = 11, $P < .001$) and glycerol production increased only 134% (241 ± 15 nmol/10^6 cells, n = 7, v 103 ± 15, n = 11, $P < .001$).

The inhibitory action of progesterone on cAMP and glycerol

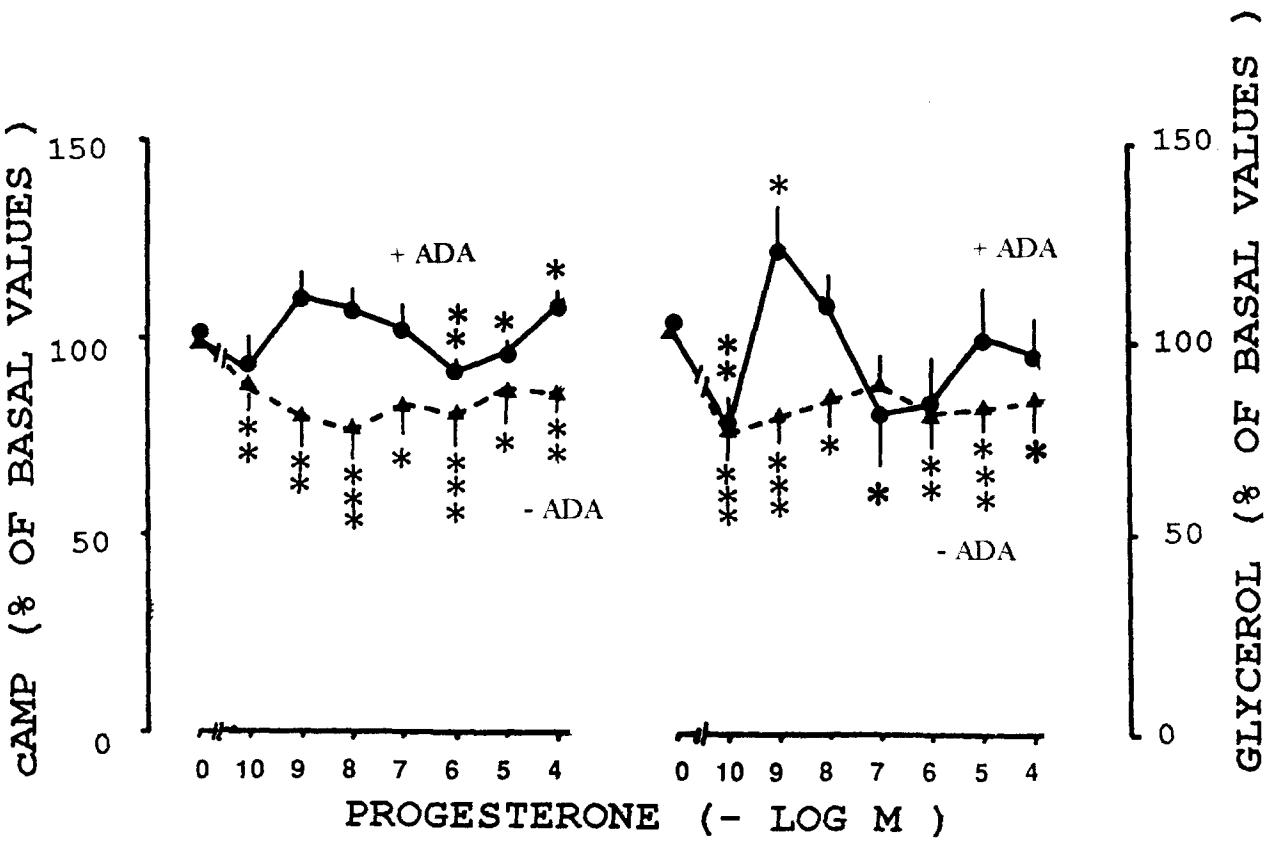

Fig 2. Dose-response curve of the progesterone effect after a 20-minute incubation on ADA-stimulated lipolysis and cAMP accumulation. (●) with ADA; (▲) no ADA. Results are expressed as a percentage of the corresponding basal value measured in the absence of progesterone. cAMP: no ADA, 18.5 ± 2.0; with ADA, 27.9 ± 2.9 pmol/10^6 cells. Glycerol: no ADA, 103 ± 15; with ADA, 236 ± 37 nmol/10^6 cells. Each point represents the mean ± SEM of 8 to 11 experiments using cells pooled from 3 to 4 rats. Progesterone effect *v* basal values (100%) (Student's *t* test): *$P < .05$, **$P < .01$, ***$P < .001$. Progesterone inhibitory effect *v* the effect of progesterone in the presence of ADA (least-squares regression analysis): cAMP, NS; glycerol, $P < .05$.

production completely disappeared in the presence of IBMX (least-squares regression analysis) (Fig 3).

Because xanthine derivatives are known to be simultaneously potent A_1 receptor antagonists and inhibitors of PDE activities,[35] we used selective A_1 receptor antagonists and PDE inhibitors in an attempt to assess the role of cAMP PDE- and A_1 receptor–mediated mechanisms in the inhibitory action of progesterone.

Action of Selective A_1 Receptor Antagonists

Two derivatives with high affinity for the A_1 receptor were studied for their influence on cAMP and glycerol levels: DPCPX 10^{-4} and 10^{-7} mol/L (K_i, 0.47 ± 2 nmol/L)[35] and CP 68,247 10^{-4} mol/L, a quinoxaline derivative (IC_{50}, 33 nmol/L).[36]

DPCPX (10^{-4} mol/L) caused a relatively smaller increase of cAMP levels (215%, 58.2 ± 10.0 pmol/10^6 cells, n = 5, *v* 18.5 ± 2.0, n = 11, $P < .001$) than IBMX (1,700%), but its effect was greater than that of CP 68,247 (158%, 47.8 ± 0.6 pmol/10^6 cells, n = 5, *v* 18.5 ± 2.0, n = 11, $P < .001$). However, the effect of DPCPX on glycerol accumulation was greater (179%, 287 ± 39 nmol/10^6 cells, n = 5, *v* 103 ± 15, n = 11, $P < .001$) than that of IBMX (134%) or CP 68,247 (102%, 208 ± 77 nmol/10^6 cells, n = 5, *v* 103 ± 15, n = 11, $P < .05$).

We also tested the effect of this xanthine at 10^{-7} mol/L, a concentration adequate to inhibit A_1 receptors while probably having little or no effect on PDEs.[37] At this low concentration, DPCPX increased cAMP levels by 101% (42.88 ± 4.9 pmol/10^6 cells *v* 21.3 ± 1.7, n = 7, $P < .001$). Glycerol levels increased only by 51% (159 ± 12 nmol/10^6 cells *v* 105 ± 6.9, n = 7, $P < .05$).

As in the presence of IBMX, 10^{-4} mol/L DPCPX abolished the inhibitory action of progesterone on glycerol and cAMP production (Fig 4), perhaps by producing an inhibition of PDE activities. On the contrary, the lower DPCPX concentration (10^{-7} mol/L, results not shown) and CP 68,247 (Fig 4), a highly selective A_1 antagonist, did not influence progesterone action (least-squares regression analysis).

PDE Inhibition

The cAMP-specific PDE family (PDE IV) is specifically inhibited by Ro 20-1724 (IC_{50}, 2 μmol/L),[37] and the Ca^{2+}-calmodulin–dependent PDE family (PDE I) by CP 41,757 (IC_{50}, 0.1 μmol/L).[36]

Ro 20-1724 (10^{-3} mol/L) increased cAMP levels by 94% (35.9 ± 9.5 pmol/10^6 cells, n = 5, *v* 18.5 ± 2.0, n = 11, $P < .05$), with a further nonsignificant increase of 72% (61.7 ± 6.9 pmol/10^6 cells, n = 5) when cells were stimulated by ADA. cAMP was increased only 57% (29.0 ± 1.9 pmol/10^6

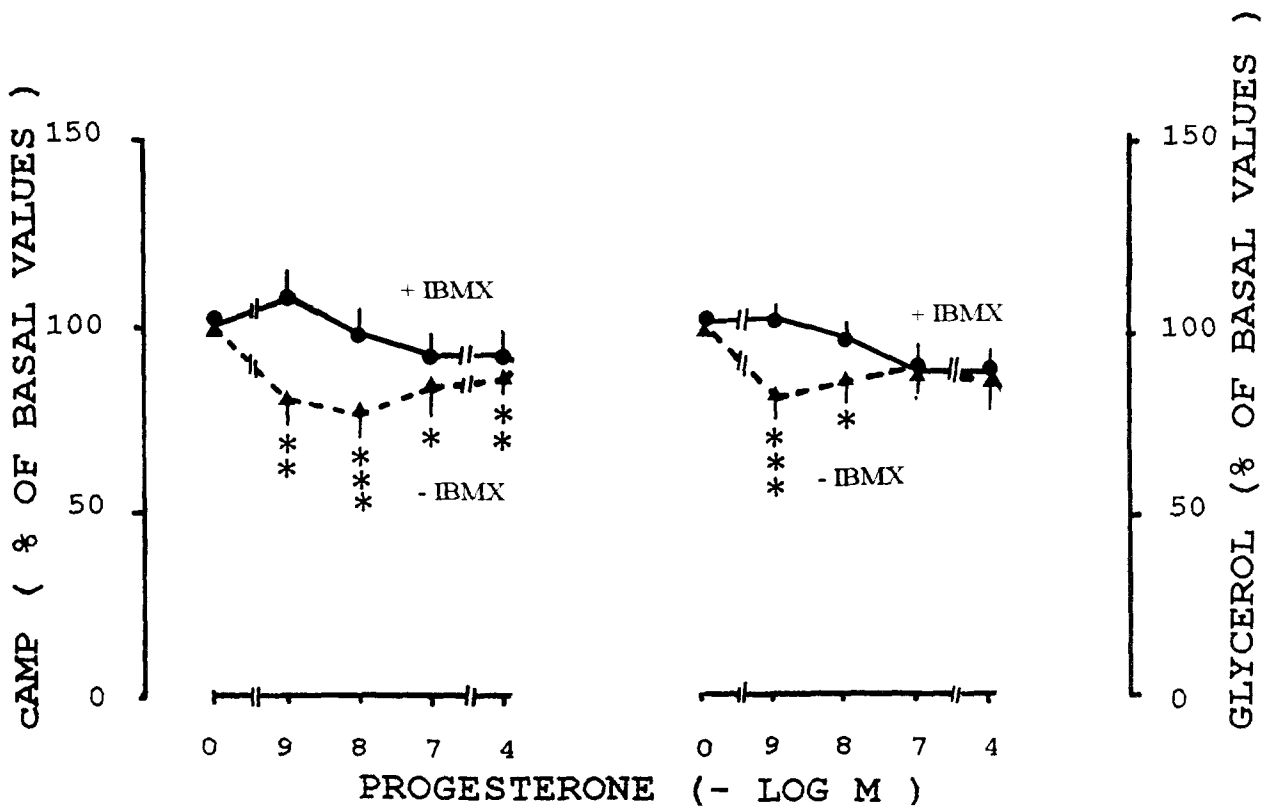

Fig 3. Dose-response curve of the progesterone effect on cAMP and glycerol accumulation in the absence (▲) or presence (●) of IBMX (10^{-3} mol/L). Results are expressed as a percentage of the corresponding basal value measured in the absence of progesterone: cAMP: absence of IBMX, 18.5 ± 2.0; presence of IBMX, 333 ± 35 pmol/10^6 cells. Glycerol: absence of IBMX, 103 ± 15; presence of IBMX, 241 ± 15 nmol/10^6 cells. Each point represents the mean ± SEM of 7 to 11 experiments using cells pooled from 3 to 4 rats. Progesterone effect *v* basal value (100%) (Student's *t* test): *$P < .05$, **$P < .01$, ***$P < .001$. Progesterone inhibitory effect on cAMP and glycerol *v* its effect in the presence of IBMX (least-squares regression analysis): $P < .05$.

cells, n = 5, *v* 18.5 ± 2.0, n = 11, $P < .01$) when CP 41,757 (10^{-4} mol/L) was used, and addition of ADA caused no further increase (16%, 33.5 ± 0.9 pmol/10^6 cells, n = 5, NS) (Fig 5).

Ro 20-1724 and CP 41,757 each increased lipolysis, the former by 62% (167 ± 28 nmol glycerol/10^6 cells, n = 5, *v* 103 ± 15, n = 11, $P < .05$) and the latter by 91% (197 ± 13 nmol/10^6 cells, n = 5, *v* 103 ± 15, n = 11, $P < .01$). In the presence of ADA, glycerol was not increased (16%, 194 ± 21 nmol/10^6 cells, n = 5, NS) by Ro 20-1724, but it was increased by CP 41,757 (28%, 252 ± 17 nmol/10^6 cells, n = 5, $P < .05$).

The inhibiting effect of progesterone on cAMP and lipolysis persisted when PDE I was blocked with CP 41,757 (least-squares regression analysis; Fig 5B). In the presence of ADA, the progesterone effect on cAMP levels disappeared but the effect on glycerol accumulation remained (results not shown).

When PDE IV was inhibited (Ro 20-1724), progesterone no longer had an effect on cAMP levels either in the absence (Fig 5A) or in the presence of ADA (results not shown). However, the steroid's effect on lipolysis in the absence of ADA remained (least-squares regression analysis; Fig 5A).

DISCUSSION

Since we have shown that progesterone inhibits glucose metabolism during a 20-minute period of incubation,[7] and since Honnor et al[21] demonstrated that there was no transient increase and decrease of A-kinase activity, which would reflect a peak of cellular cAMP concentrations, and examined A-kinase activity and glycerol release at a single time (25 minutes), we chose to determine both glycerol and cAMP release after a 20-minute incubation period. Further, because Schwabe et al[38] found that the responsiveness of adipocytes to a given hormonal stimulator was highly dependent on the concentration of cells in the incubation medium, we were careful to use an equal number of fat cells in the various experiments.

Because triacylglycerol hydrolysis occurs in unstimulated adipocytes,[39] we studied first the effects of progesterone in this system. The determined basal release of glycerol is of the same order of magnitude as that reported by others.[40] In the ensuing experiments, the effects of progesterone in unstimulated cells were compared with those in cells with cAMP levels augmented by isoproterenol, ADA, inhibitors of PDEs, etc.

Our results strongly indicate that physiologic concentrations of progesterone added to isolated adipocytes increase the release of adenosine, with the maximal effect occurring by the end of the 20-minute incubation, as shown by others.[41] Released adenosine is sufficient to modify cAMP levels and consequently the lipolytic process; it inhibits basal lipolysis, as previously described.[41] Thus, in our experiments, the release of adenosine coincides with the process of progesterone stimulation of fat

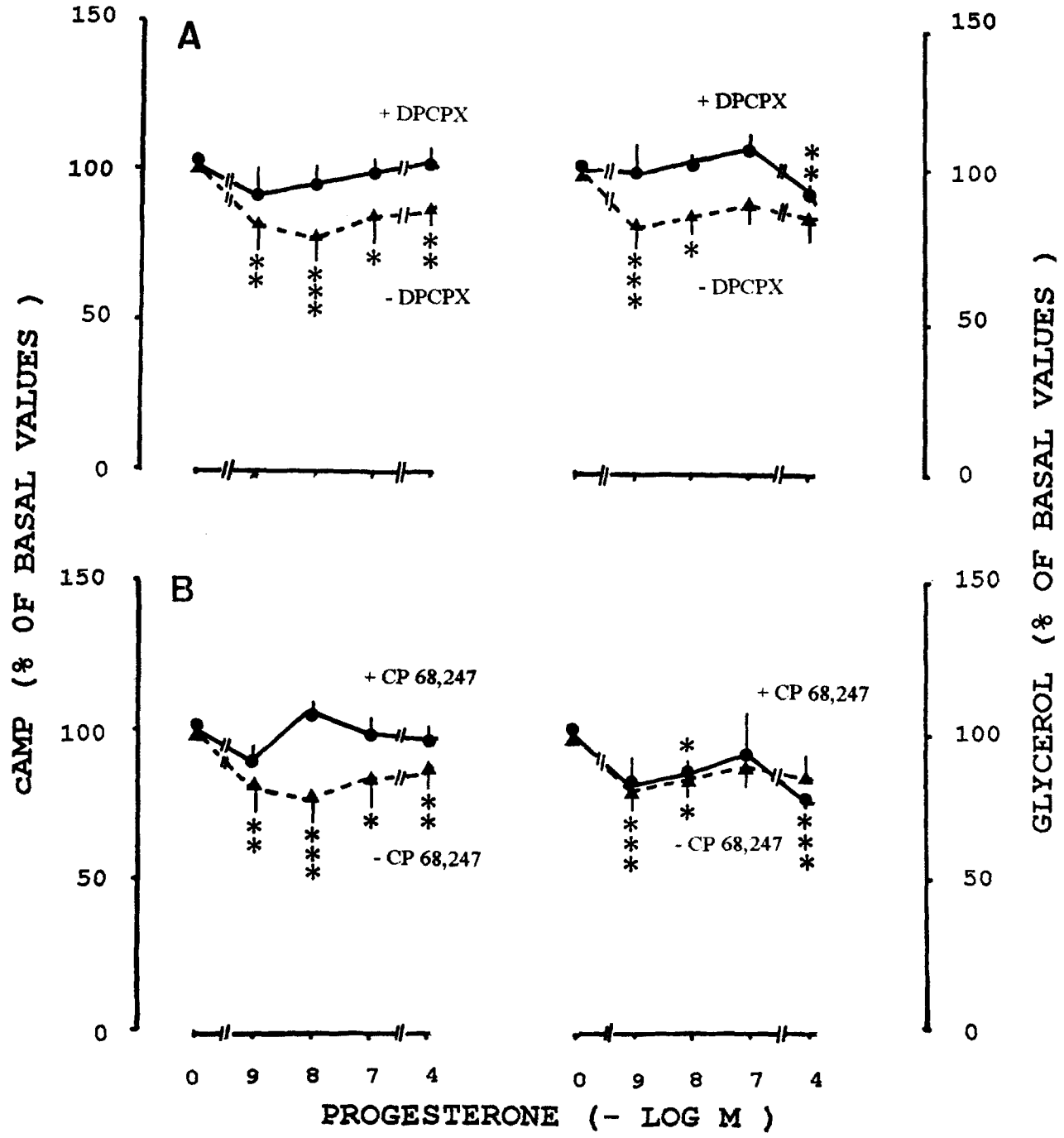

Fig 4. Dose-response curve of the progesterone effect on cAMP and glycerol accumulation in the absence (▲) or presence (●) of the 2 A_1 receptor antagonists: (A) DPCPX (10^{-4} mol/L) and (B) CP 68,247 (10^{-4} mol/L). Results are expressed as a percentage of the corresponding basal value measured in the absence of progesterone. (A) cAMP: absence of DPCPX, 18.5 ± 2.0; presence of DPCPX, 58.2 ± 10 pmol/10^6 cells. Glycerol: absence of DPCPX, 103 ± 15; presence of DPCPX, 287 ± 39 nmol/10^6 cells. (B) cAMP: absence of CP 68,247, 18.5 ± 2.0; presence of CP 68,247, 47.8 ± 0.6 pmol/10^6 cells. Glycerol: absence of CP 68,247, 103 ± 15; presence of CP 68,247, 208 ± 77 nmol/10^6 cells. Each point represents the mean ± SEM of 5 to 11 experiments using cells pooled from 3 to 4 rats. Progesterone effect *v* basal value (100%) (Student's *t* test): *$P < .05$, **$P < .01$, ***$P < .001$. Progesterone inhibitory effect on cAMP and glycerol *v* its effect in the presence of DPCPX: $P < .05$; in the presence of CP 68,247: NS (least-squares regression analysis).

cells and may explain the decline of cAMP levels and of lipolysis observed after hormonal stimulation.

The inhibitory effect of progesterone on both cAMP levels and lipolysis of unstimulated cells persisted after stimulation with isoproterenol. ADA, added to unstimulated cells to destroy endogenously produced adenosine, decreased the inhibitory action on lipolysis but had no effect on cAMP accumulation. However, removal of endogenous adenosine in isoproterenol-

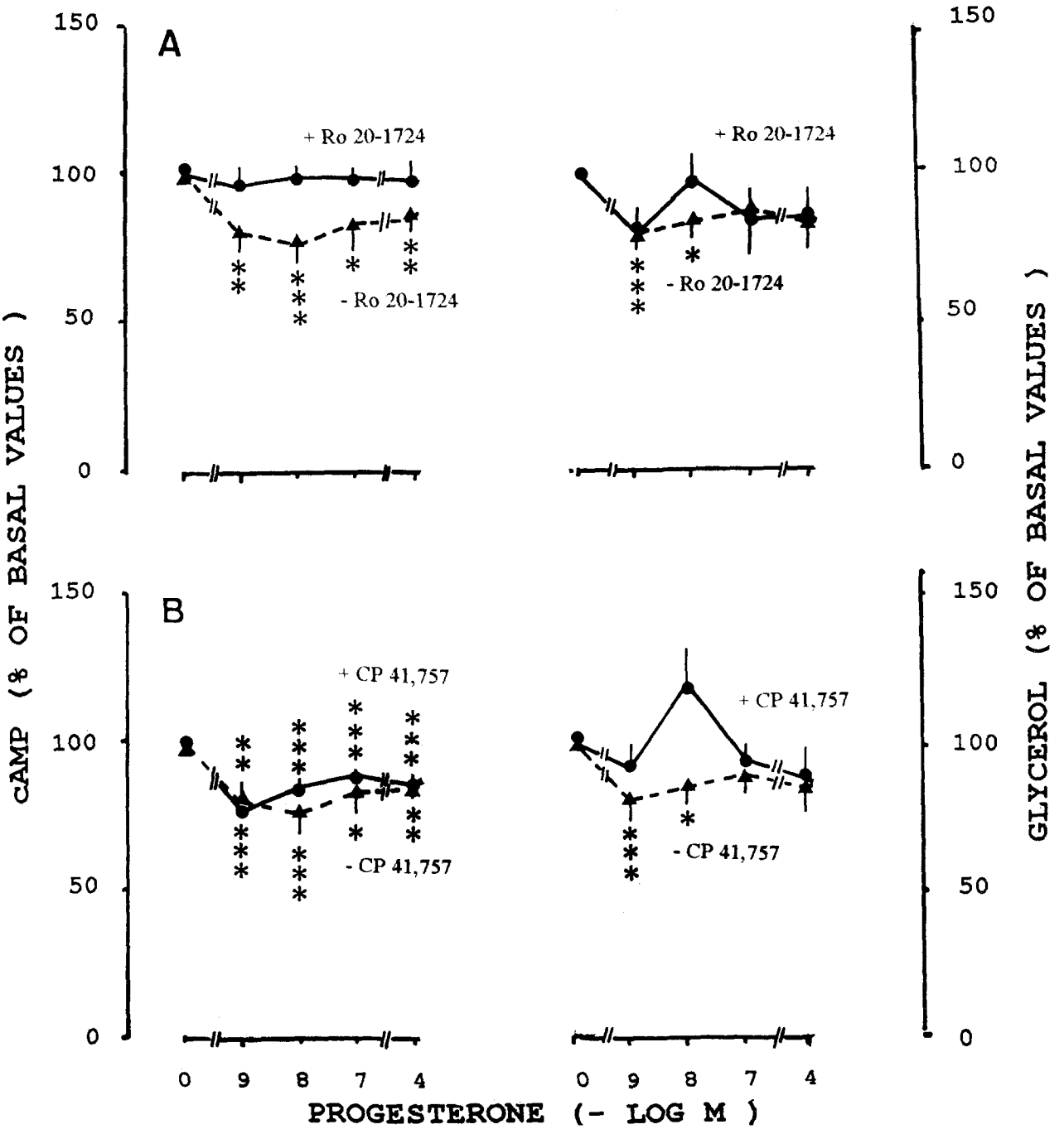

Fig 5. Dose-response curve of the progesterone effect on cAMP and glycerol accumulation in the absence (▲) or presence (●) of the 2 PDE inhibitors: (A) Ro 20-1724 10^{-3} mol/L (PDE IV family) and (B) CP 41,757 10^{-4} mol/L (PDE I family). Results are expressed as a percentage of the corresponding basal value measured in the absence of progesterone. (A) cAMP: absence of Ro 20-1724, 18.5 ± 2.0; presence of Ro 20-1724, 35.9 ± 9.5 pmol/10^6 cells. Glycerol: absence of Ro 20-1724, 103 ± 15; presence of Ro 20-1724, 167 ± 28 nmol/10^6 cells. (B) cAMP: absence of CP 41,757, 18.5 ± 2; presence of CP 41,757, 29.0 ± 1.9 pmol/10^6 cells. Glycerol: absence of CP 41,757, 103 ± 15; presence of CP 41,757, 197 ± 13 nmol/10^6 cells. Each point represents the mean ± SEM of 5 to 11 experiments using cells pooled from 3 to 4 rats. Progesterone effect *v* basal value (100%) (Student's *t* test): *$P < .05$, **$P < .01$, ***$P < .001$. Progesterone inhibitory effect on cAMP and glycerol *v* its effect in the presence of Ro 20-1724: cAMP, $P < .05$; glycerol, NS; in the presence of CP 41,757: NS (least-squares regression analysis).

stimulated cells completely blocked the progesterone inhibitory effect both on cAMP and on glycerol accumulation. These findings suggest that the mechanism of the progesterone-induced decrease in cAMP is mediated by adenosine at high but not at low cAMP levels, and that this mechanism may be different from the mechanism regulating lipolysis. The inhibitory effect of progesterone on lipolysis persisted in the presence of high levels of cAMP produced by isoproterenol, suggesting that this effect is mediated by adenosine, whatever the level of cAMP.

It has been reported that adenosine, by reacting with a membrane-bound receptor A_1,[42,43] reduces the accumulation of cAMP in adipocytes by inhibiting adenylylcyclase activity[44] through a transducing G_i protein,[45,46] and on the other hand by increasing activities of PDEs.[47,48]

Since progesterone has no direct effect on cyclase activity,[24,49] it may decrease cAMP levels by either of these pathways.

The xanthines represent a potent class of adenosine receptor antagonists; however, some of them are capable of inhibiting cAMP breakdown by PDEs.[31]

The inhibitory effect of progesterone on cAMP and glycerol accumulation was counterbalanced both by IBMX, which inhibits both soluble and particulate cAMP PDE[48] but is also a potent antagonist of adenosine receptors,[37] and by the more selective A_1 antagonist, DPCPX (10^{-4} mol/L). DPCPX at 10^{-4} mol/L may also induce a minor inhibition of PDE activity.[50] Yet 10^{-7} mol/L DPCPX is considered a concentration typical for signaling solely via the adenosine A_1 receptor, antagonizing up to 10^{-7} mol/L adenosine,[51] as is CP 68,247, a highly selective A_1 antagonist.[36] Although each of these antagonists increase basal cAMP and glycerol levels, they have no effect on the inhibitory actions of progesterone. These results suggest that adenosine A_1 receptors are not directly involved in the progesterone inhibitory effect.

R-PIA, an adenosine analog that interacts with A_1 receptors, decreases both cAMP and glycerol levels in the presence of ADA. Combining progesterone and R-PIA produced no further decrease of this effect. In addition, the A_1 receptor antagonists block the inhibitory effects of R-PIA over the same concentration ranges that affect progesterone action. These actions may suggest, on the contrary, an effect of progesterone on A_1 receptors. However, it was shown that R-PIA activates the particulate low-K_m PDE (PDE III), whereas soluble cAMP-PDE activity (PDE IV) was not altered.[52] So an action of R-PIA and progesterone on different PDE activities cannot be excluded, all the more so since the effects of PIA on particulate PDE activity persist for at least 30 minutes. Postreceptor mechanisms, mediated via guanyl nucleotide-binding proteins, might be implicated in these effects.[52]

Regarding the possible effect of progesterone on PDE activities, experiments were performed in the presence of specific inhibitors. At least three different families of cyclic nucleotide PDEs are known in adipocytes: the cGMP-inhibited PDE family (PDE III), the cAMP-specific PDEs (PDE IV), and the Ca^{2+}-calmodulin–dependent family (PDE I). The PDE III family, associated with the particulate fractions from rat adipocytes, is activated in response to insulin or agents that increase cAMP,[53] whereas cAMP-specific PDEs (soluble fractions) (PDE IV) exhibit greater than a 50-fold selectivity for cAMP as substrate.[54]

Since progesterone action in adipocytes was mediated by Ca^{2+}-calmodulin–dependent mechanisms[24] and, as shown earlier, induced a decrease in cAMP accumulation, we studied the effects of progesterone on PDE I and PDE IV activities. These activities are inhibited by two specific PDE inhibitors. One of these, Ro 20-1724, does not interact with the binding of adenosine to A_1 receptors[55] and is a more potent inhibitor of soluble (PDE IV) than of particulate (PDE III) cAMP-PDE,[28] which can be antagonized with an IC_{50} of 190 μmol/L.[48] Another PDE inhibitor, CP 41,757, is a specific inhibitor of the Ca^{2+}-calmodulin–dependent PDE family (PDE I).[36] As expected, these two compounds increased cAMP accumulation and lipolysis in the absence of progesterone. However, only the effect of the PDE I inhibitor was further increased by ADA, in good agreement with the findings of Yeager et al.[56]

The response of adipocytes to progesterone in the presence of PDE inhibitors was studied. Inhibition of PDE I did not affect lipolysis or cAMP levels when progesterone was present, but the cAMP response disappeared when endogenous adenosine was hydrolyzed, suggesting that PDE I may not be implicated in the inhibitory actions of progesterone. Ro 20-1724 abolishes the action of progesterone to decrease cAMP in the absence or presence of endogenous adenosine; the inhibitory effect of the steroid on lipolysis persisted, but only in the presence of endogenous adenosine. These results are consistent with those reported by Elks and Manganiello[28] showing that inhibition of soluble PDE (PDE IV) might result in larger increases in total cAMP content with smaller effects on lipolysis. In addition, the effects of progesterone on lipolysis differ from those on cAMP accumulation, suggesting that the progesterone inhibitory action on lipolysis, which is not directly related to cAMP changes, may be modulated by adenosine and/or the particulate PDE (PDE III), particularly since Teo et al[47] found that adenosine activates a particulate cAMP-PDE.

It has been recently reported that changes in cAMP-dependent protein kinase rather than cAMP are better correlated with lipolysis.[21] However, one cannot exclude other factors involved in the regulation of lipolysis; for example, Ca^{2+}[51,57,58] may be implicated in the effect of the steroid.[24]

Our data suggest that although progesterone increases adenosine release, its inhibitory effects on lipolysis and on cAMP levels are not mediated by binding to the A_1 adenosine receptor, with the action of the steroid persisting in the presence of specific A_1 receptor antagonists. Moreover, PDE IV seems involved in the effect of the steroid on cAMP concentrations, but not on lipolysis; the latter may be modulated by the increased release of adenosine, perhaps by an effect on PDE III activity. PDE I seems not to be implicated. Further experiments to assess a possible direct effect of progesterone on inhibitory G_i proteins in relation or not with an effect on cAMP PDE activities, perhaps activated by adenosine, are needed to shed more light on the present results.

ACKNOWLEDGMENT

The authors are grateful to Professor L. Rosenberg, University of California, Berkeley, for a critical review of the manuscript and helpful suggestions and corrections. We also thank L. Duvert for skilled technical assistance and M.H. Davant for typing the manuscript. We are indebted to Dr Deraedt from Roussel-Uclaf (Romainville, France) for the generous gift of progesterone. We also acknowledge Hoffman La Roche (Basel, Switzerland) for the gift of Ro 20-1724, and Pfizer for providing us with CP 68,247 and CP 41,757.

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