HORMONAL CONTROL OF PORCINE ADIPOSE TISSUE FATTY ACID RELEASE AND CYCLIC AMP CONCENTRATION


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ABSTRACT

Compared with adipose tissue from other mammals, porcine adipose tissue has stringent specificity for stimulation of lipolysis by analogs of norepinephrine. This study was to ascertain whether the specificity for control was reflected in the concentration of tissue cyclic AMP (cAMP). Adipose tissue slices were incubated and concentrations of tissue cAMP and free fatty acids (FA) released to the medium were measured. It was necessary to include theophylline in the incubation medium to be able to measure changes in cAMP concentration. Fatty acid release and cAMP production were increased most effectively by the β-adrenergic agonists; isoproterenol, fenoterol, dobutamine and the mixed α + β-adrenergic agonist, epinephrine. Isoproterenol-stimulated FA and cAMP production were both inhibited by the β-adrenergic antagonist, propranolol. There was no evidence for α2-adrenergic inhibition of lipolysis in porcine adipose tissue because clonidine (α2-adrenergic agonist) did not lower isoproterenol-induced FA or cAMP levels and phentolamine, an α-adrenergic antagonist, did not increase epinephrine-stimulated FA release or cAMP generation. These results imply that the stringent specificity observed for stimulation of swine adipose tissue lipolysis resides in the β-adrenergic receptor coupled to cAMP production.

(Key Words: Pigs, Lipid Metabolism, Lipolysis, Adipose Tissues, Catecholamines.)

Introduction

It is generally accepted that the lipolytic response of adipose tissue to a variety of hormones is mediated by a cascade-type regulatory system. The initial event is hormone interaction with specific membrane receptors followed by activation of adenylate cyclase. Elevated adenylate cyclase activity increases the concentration of cyclic-AMP (cAMP), the intracellular second messenger, which activates a protein kinase that, in turn, phosphorylates and activates a triacylglycerol lipase. The lipase catalyzes the degradation of triacylglycerol to free fatty acid (FA) and glycerol (Steinberg, 1976; Fain and Garcia-Sainz, 1983).

Because many norepinephrine analogs stimulate adipose tissue lipolysis in a variety of species but only a few analogs stimulate porcine adipose tissue lipolysis (Mersmann, 1984a,b,c), it was of interest to ascertain whether the specificity in porcine adipose tissue resides in the receptor or at some other point in the activation cascade. We assessed the capacity of norepinephrine analogs to elevate porcine adipose tissue cAMP concentrations to provide evidence that the specificity resides in the capacity of the coupled receptor-adenylate cyclase to produce cAMP.

Methods and Materials

Crossbred (1/4 Yorkshire, 1/4 Landrace, 1/4 Large White, 1/4 Chester White) female pigs were raised under normal husbandry conditions and fed ad libitum a corn-soybean meal-based diet (16% crude protein) from 10 wk of age.
The pigs (30 to 55 kg) were anesthetized with sodium thiopental and adipose tissue biopsy samples were obtained from the dorsal neck region with a biopsy gun, as described previously (Mersmann, 1983). The adipose tissue was placed in .9% NaCl containing 5.6 mM glucose at about 35 C for transport to the laboratory. A pool of tissue slices, .4 mm thick, was prepared (about 15 min after biopsy) from the biopsy samples from each pig.

To measure the lipolytic rate, tissue slices (100 mg total) were incubated for 120 min in Krebs-Ringer bicarbonate buffer containing 4% bovine serum albumin\(^7\), 5.6 mM glucose and .56 mM ascorbate, unless indicated otherwise. The medium was gassed with 5% CO\(_2\) in O\(_2\). Incubations were in sealed flasks in an atmosphere of 5% CO\(_2\) in O\(_2\) at 37 C. Basal lipolytic rates were determined in triplicate without addition of exogenous hormones\(^8\). Stimulated lipolytic rates were determined in triplicate in the presence of an exogenous norepinephrine analog, e.g., \(10^{-4}\) M isoproterenol, as indicated for each experiment. Except for the time course without theophylline in figure 1, all flasks, including those for basal rate measurement, contained \(10^{-3}\) M theophylline. The lipolytic rate was assessed by extraction (Dole and Meinertz, 1960) and titration (Kelley, 1965) of FA concentration in the medium. Details for measurement of lipolytic rates have been indicated previously (Mersmann et al., 1974; Mersmann, 1984a; Mersmann and Hu, 1987). It should be noted that measurement of FA release to the medium is not strictly a measurement of the lipolytic rate because it is confounded by an accompanying and unknown rate of re-esterification. Past experience with porcine adipose tissue lipolytic systems indicates that assessment of medium glycerol usually produces the same pattern for lipolysis as measurement of medium FA, but in itself is no panacea. The ratio of FA to glycerol release may be from 1 to 2.8 in the presence of many substances, implying re-esterification rates of large to small magnitude, respectively. The ratio also may be greater than 3, implying incomplete hydrolysis of the triacylglycerol molecule (Mersmann et al., 1974, 1975, 1976a; Mersmann, 1986b).

To measure changes in tissue cAMP concentration, 100-mg tissue slices were incubated for 3 min at 37 C in media identical to that used to measure lipolytic rates, unless indicated otherwise. Isolation and assay of cAMP were according to Gilman (1970) with the following modifications. Tissue from an incubation flask was homogenized\(^9\) in 3 ml of cold 5% trichloroacetic acid. Homogenates were centrifuged at 15,000 x g for 10 min at 0 to 4 C; supernates were filtered through two layers of cheese cloth to remove floating fat and then were extracted six times with 8 ml diethyl ether to remove the acid. Finally, extracts were lyophilized and redissolved in .8 ml of 50 mM sodium acetate. The competitive binding assay contained in a final volume of 300 ~\(\mu\)l: 20 ~\(\mu\)l of 50 mM sodium acetate, pH 4.0, 20 ~\(\mu\)l of inhibitor protein (2.0 mg·mF\(^-1\)), 10 ~\(\mu\)l of H\(^3\)-cAMP (about .65 pmol, 15,000 cpm)\(^10\) and standards (.2 to 20 pmol cAMP) or samples in 200 ~\(\mu\)l volume. Fifty ~\(\mu\)l of binding protein (.25 mg·mF\(^-1\)) in .2% bovine serum albumin\(^11\) was added to initiate the reaction. Samples were incubated at 4 C for 24 h. At the end of the incubation, the samples were filtered and washed three times with 20 mM potassium phosphate buffer (pH 6.0).\(^12\) The bound H\(^3\)-cAMP trapped on the filter was counted in a liquid scintillation counter. Binding protein and protein kinase inhibitor used in the cAMP assay were isolated from fresh bovine muscle according to Gilman and Murad (1975).

Statistical analyses were conducted by the General Linear Model Procedure of SAS (1982). Data were analyzed by analysis of variance with

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\(^7\) Fraction V, fatty acid poor (A 6003), Sigma Chemical Co., St. Louis, MO 63178.

\(^8\) The following companies generously supplied the adrenergic agonists used: Boehringer-Ingelheim Ltd., Ridgefield, CT 06877 (clonidine·HCl, fenoterol·HBr); Ciba-Geigy Corp., Summit, NJ 07901 (phenolamine·HCl); Pfizer Inc., Groton, CT 06340 (quinoterol·2HCl); Philips-Roxane Inc. (now Boehringer-Ingehelm Animal Health, Inc.), St. Joseph, MO 64502 (clenbuterol·HCl); Syntex Corp., Palo Alto, CA 94304 (tazolol·HCl); and Lilly Research Laboratories, Greenfield, IN 46140 (crystalline porcine insulin (SF144-2A) and dobutamine-HCl). Special chemicals purchased from Sigma Chemical were L-epinephrine bitartrate, L-isoproterenol bitartrate, DL-propranolol·HCl and theophylline.

\(^9\) Polytron Homogenizer, Brinkman Instruments Co., Westbury, NY 11590.

\(^10\) Adenosine 5'-cyclicphosphate, diammonium salt [2, 8-\(^3\)H] (NET-275), NEN Research Products, Boston, MA 02118.

\(^11\) Fraction V (A4503), Sigma Chemical Co., St. Louis, MO 63178.

\(^12\) Filters were .45 ~\(\mu\)m (HAWP 025); Millipore Corp., Bedford, MA 01730.
Dunnett's procedure (Steele and Torrie, 1960) to separate the means of treatment from the control.

Results and Discussion

Incubation Conditions. In contrast to results with hamster (Hittelman et al., 1973) and rat (Allen, 1985) adipose tissue, isoproterenol-stimulated porcine adipose tissue did not produce a measurable increase in tissue cAMP concentration in the absence of theophylline, a phosphodiesterase inhibitor (figure 1). Theophylline may act as a phosphodiesterase inhibitor to enhance effectively the cAMP produced or as an inhibitor of adenosine receptors to decrease the inhibition by endogenously produced adenosine (Hjemdahl and Fredholm, 1976). We would speculate that the former mechanism is operative in the current experiments because theophylline stimulated FA release in the presence of $10^{-4}$ isoproterenol (figure 1), a concentration expected to be saturating (Mersmann et al., 1974), whereas adenosine removal with adenosine deaminase made the tissue more sensitive to epinephrine but did not change the maximal FA release (Mersmann, 1984c). In the presence of isoproterenol plus theophylline, cAMP accumulated rapidly and concentration remained elevated through 120 min of incubation. The increase in FA release in the presence of isoproterenol was observable at 20 min and continued through 120 min of incubation; the lag period for release of FA during stimulation by isoproterenol has been observed, routinely, for porcine adipose tissue in this laboratory (Mersmann and Hu, 1987). The rapid increase in cAMP concentration, its maintenance with time of incubation and the lag in FA release are similar to observations in rat adipose tissue (Allen, 1985). Stimulation of FA release by isoproterenol plus theophylline was greater than by isoproterenol alone (figure 1), as demonstrated previously (Mersmann et al., 1974, 1976b; Mersmann, 1984a,b,c). The current results clearly indicated that theophylline must be present in the incubation medium to detect changes in cAMP concentration. On the basis of this time course we chose 3- and 120-min incubation periods to measure changes in concentration of cAMP in the tissue and FA in the medium.

The response of porcine adipose tissue to stimulation by the $\beta$-adrenergic agonist, isopro-

![Figure 1](image.png)

Figure 1. Effect of incubation time on FA release and cAMP concentration. Assay was in triplicate flasks with 100 mg tissue in Krebs-Ringer-bicarbonate buffer containing 5.6 mM glucose, .56 mM ascorbate and 4% bovine serum albumin. Stimulated lipolytic rate was in presence of $10^{-4}$ M isoproterenol with or without $10^{-3}$ M theophylline (Theo). The net stimulated rate (stimulated minus basal) is reported. The ordinate unit is $\mu$eq FA-120 min$^{-1}$·g$^{-1}$. Data are means from two to four different experiments, each using tissue from a different pig. Selected vertical bars representing standard deviations are presented.
terenol, was measured using a dose x response curve generated with isoproterenol concentrations varied by orders of magnitude from $10^{-9}$ to $10^{-4}$ M (figure 2). Isoproterenol increased release of FA about 13-fold above the basal condition ($10^{-3}$ M theophylline but no isoproterenol), whereas cAMP concentration in the tissue increased <50%. The small but consistent increase in cAMP concentration suggests a selected pool, rather than the total cellular pool of cAMP functions in the lipolytic cascade. Maximal FA release and cAMP concentration were produced at about $10^{-7}$ M isoproterenol. Because of the extreme variability between animals in response to a lipolytic agonist (Mersmann et al., 1974), and because there is no evidence for inhibition at higher agonist concentrations (figure 2 and Mersmann et al., 1974), $10^{-5}$ or $10^{-4}$ M isoproterenol was used in most other studies to achieve maximal FA release and cAMP concentration.

Adrenergic Effectors. Previous studies indicated stringent specificity of norepinephrine analogs for release of FA to the medium by porcine adipose tissue (Mersmann, 1984b). Selected agonists used in those studies (Mersmann, 1984b) were tested for effects on tissue cAMP concentration. The agonist concentration used was selected from the dose x response curves previously generated (Mersmann, 1984b), and was such that Vmax would be approximated for the lipolytic response. Theophylline was present in all flasks so that cAMP concentration could be measured. Isoproterenol at $10^{-5}$ M stimulated the release of FA to the medium about 10-fold compared with the basal condition ($10^{-3}$ M theophylline only), whereas cAMP concentration in the tissue was stimulated about twofold (figure 3). Fenoterol, a purported $\beta_2$-adrenergic agonist, stimulated FA release to the medium to about the same extent as isoproterenol, as observed in previous studies (Mersmann, 1984a,b); fenoterol increased cAMP concentration also (figure 3). Quinterenol, another purported $\beta_2$-agonist, was considered not active regarding lipolytic stimulation in

Figure 2. Effect of isoproterenol on FA release and cAMP concentration. Incubation was similar to figure 1 except all flasks contained $10^{-3}$ M theophylline and isoproterenol varied by orders of magnitude. Incubation times were 120 min and 3 min for FA release and tissue cAMP concentration, respectively. Data are means from three different experiments, each using tissue from a different pig. Vertical bars are standard deviations. Plots of log dose x percent response yielded ED_{50} of about $10^{-9}$ M for cAMP concentration and $5 \times 10^{-9}$ M for FA release (data not indicated).
Figure 3. Effect of various lipolytic and antilipolytic agents on isoproterenol-stimulated FA release and cAMP generation. Incubation as in figure 1 except different agonists replaced isoproterenol. All flasks contained 10^-3 M theophylline. (The basal flasks contained theophylline only.) Additions to flasks were 10^-5 isoproterenol (Iso, β1 + β2-agonist); 2.6 × 10^-4 M fenoterol (Fen, β2-agonist); 3.1 × 10^-4 M quinterenol (Quin, β2-agonist); 3.4 × 10^-5 M propranolol (Prop, β-antagonist) or 3.8 × 10^-5 M clonidine (Clon, α2-agonist). Stimulation of FA release was greater (P<.05) than the basal response in the presence of Iso and Fen but not Quin. Production of cAMP was stimulated (P<.01) by Iso and Fen, but was lower (P<.01) than the basal rate for Quin. Concentrations of FA and cAMP elevated by Iso were inhibited (P<.05) by Prop but not by Clon. Data are means from three different experiments, each using tissue from a different pig.

Porcine adipose tissue (Mersmann, 1984b) because it was not possible to demonstrate a dose × response relationship. Quinterenol did not increase the release of FA to the medium in the present experiment at a single high dose. Quinterenol did not cause an increase in cAMP concentration (figure 3), but lowered it compared with the basal level (observed in all three pigs and inexplicable at this time). The same specificity for these purported β2-adrenergic agonists was observed in vivo also, because intravenous infusion of isoproterenol and fenoterol into pigs caused an acute increase in plasma FA concentration, whereas infusion of quinterenol did not (Mersmann, 1987).

When tissue was incubated with isoproterenol plus propranolol, a β-adrenergic agonist and antagonist, respectively, FA release to the medium and increase in tissue cAMP concentration were both completely inhibited (figure 3). Propranolol repeatedly has been shown to inhibit isoproterenol-stimulated rates of porcine adipose tissue lipolysis in vitro (Mersmann et al., 1974; Mersmann, 1984a,b), and intravenous infusion of propranolol inhibited the isoproterenol-stimulated increase in plasma FA (Mersmann, 1987). Stimulation of α2-adrenergic receptors in adipose tissue would be expected to inhibit cAMP production and, consequently, inhibit FA release (Fain and Garcia-Sainz, 1983; Lafontan et al., 1983). However, there is no evidence for α2-adrenergic inhibition of swine adipose tissue lipolysis, implying that α2-receptors are not present on porcine adipocytes or that stimulation of the receptors does not inhibit lipolysis in swine adipose tissue (Mersmann, 1984c). Isoproterenol-stimulated rate of lipolysis and production of cAMP was not inhibited by the α2-adrenergic agonist, clonidine (figure 3). Likewise, intravenous clonidine infusion did not lower plasma FA concentration elevated by isoproterenol infusion in vivo (Mersmann, 1987).

Two purported β1-adrenergic agonists were examined for their ability to increase cAMP concentration. One agonist, dobutamine, modestly stimulated porcine adipose tissue lipolysis (Mersmann, 1984b) and this was reflected after in vivo infusion by elevation of plasma FA (Mersmann, 1987). Tazolol was inactive in vitro...
(Mersmann, 1984b) and in vivo (Mersmann, 1987). In the present study, dobutamine stimulated FA release to the same extent as isoproterenol and dobutamine elevated tissue cAMP concentration (figure 4). The larger stimulation of FA release by dobutamine observed in the present experiment compared with the previous study (Mersmann, 1984b) probably resulted from the presence of theophylline in the current but not in the previous experiments. Tazolol did not elevate FA in the medium or tissue cAMP concentration (figure 4). Clenbuterol, another purported β₂-adrenergic agonist, did not stimulate FA release in vitro (figure 4 and Mersmann, 1987), and it did not cause an increase in cAMP concentration (figure 4). Intravenous infusion of clenbuterol into pigs elevated plasma FA concentration (Mersmann, 1987), suggesting a mechanism in vivo other than interaction with the adipocyte β-adrenergic receptor.

Epinephrine, an α- plus β-adrenergic agonist stimulated FA release, as reported previously (Mersmann et al., 1974), and increased tissue cAMP concentration (figure 4). The addition of phentolamine, an α-adrenergic antagonist (figure 4), did not further enhance the epinephrine-stimulated FA release (as observed previously, Mersmann, 1984c) or tissue cAMP concentration. Similar results were observed in vivo; epinephrine infusion elevated plasma FA concentration but simultaneous infusion of phentolamine did not enhance the epinephrine effect (Mersmann, 1987). If α-adrenergic agonists caused an inhibition of lipolysis in swine adipose tissue, the response to epinephrine, an agonist with both α- and β-adrenergic activity, would be enhanced in the presence of the α-antagonist, phentolamine. The lack of response of tissue cAMP concentration to phentolamine provides further evidence that α₂-adrenergic receptors either are not present on or do not mediate inhibition of lipolysis in swine adipocytes.

In the absence of theophylline, insulin inhibits the porcine adipose tissue-stimulated lipolytic rate (Mersmann, 1986a; Mersmann and Hu, 1987) but does not change the basal lipolytic rate in vitro (Walton and Etherton, 1986; Mersmann and Hu, 1987). Also, intravenous insulin infusion decreases the plasma FA concentration (Mersmann, 1986a). Insulin does not seem to affect adenylate cyclase activity directly (Steinberg, 1976). Therefore, it appears that the antilipolytic action of insulin may result from stimulation of cAMP phosphodiesterase, resulting in decreased tissue concentration of cAMP and consequently a reduced phosphorylation state and activity of hormone sensitive lipase (Lafontan and Berlan, 1985).

![Figure 4](image-url)  
Figure 4. Response to various adrenergic agonists and antagonists. Incubation as in figure 3. Additions to flasks were 10⁻⁵ M isoproterenol (Iso, β-agonist); 3 × 10⁻⁵ M dobutamine (Dob, β₁-agonist); 3.9 × 10⁻⁴ M tazolol (Taz, β₂-agonist); 3.2 × 10⁻⁴ M clenbuterol (Clen, β₂-agonist); 10⁻⁴ M epinephrine (Epi, α+β agonist) or 10⁻⁵ M phenolamine (Phen, α-antagonist). Release of FA and cAMP concentration were stimulated (P<.05) by Dob and Epi but not (P>.05) by Taz and Clen. Phenolamine did not enhance the agonistic Epi effect. Vertical bars indicate pooled SE for FA and cAMP.
Because theophylline probably inhibits cAMP phosphodiesterase (Wong et al., 1985), insulin would not be expected to stimulate the enzyme and thus inhibit lipolysis in the presence of theophylline. Because no change in cAMP can be demonstrated in the absence of theophylline, and because at the present, no change in cAMP concentration can be measured in the presence of insulin (data not indicated), it is not known whether insulin acts by depression of tissue cAMP concentration in porcine adipose tissue. In rat adipose tissue (Khoo et al., 1973), protein kinase activation and glycerol release are diminished by insulin, whereas cAMP concentration assayed in the presence of theophylline is marginally decreased.

Conclusion

The results presented in this paper suggest that the stringent specificity observed for stimulation of swine adipose tissue lipolysis in vitro (Mersmann, 1984b) by analogs of norepinephrine resides in the β-adrenergic receptor coupled to cAMP production. Because an α-adrenergic agonist and antagonist did not decrease or increase cAMP tissue concentration, respectively, additional evidence was provided for support of the concept that porcine adipose tissue lipolytic activity is not inhibited by α2-adrenergic receptors.

Literature Cited


