Prolonged In Vitro Exposure of Broiler Adipocytes to Somatostatin Enhances Lipolysis and Induces Desensitization of Antilipolysis

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ABSTRACT Adipocytes isolated from abdominal fat of female broilers and maintained in primary culture were used to characterize acute and chronic effects of somatostatin (SRIF) on lipolysis and antilipolysis and to determine whether desensitization and cross-regulation phenomena are involved in the regulation of adipocyte metabolism by SRIF and glucagon. Acute exposure of adipocytes to SRIF resulted in a dose-dependent inhibition of basal and glucagon-stimulated lipolysis. The potency and extent of lipolysis inhibition by SRIF were inversely related to the dose of glucagon used to stimulate lipolysis. Preincubation of adipocytes with SRIF induced a dose-response and time-dependent increase in the set-point of lipolysis. Adipocyte sensitivity to glucagon was not altered by SRIF pretreatment. In contrast, preincubation with SRIF reduced adipocyte sensitivity and maximal responsiveness to SRIF. The pattern and extent of antilipolysis attenuation were dependent on the dose and time of preincubation with SRIF as well as on the concentration of glucagon used to acutely stimulate lipolysis. In two experiments, the attenuation of antilipolysis induced by SRIF pretreatment was observed in the absence of enhanced lipolysis, whereas in one experiment enhanced lipolysis was observed in the absence of attenuated antilipolysis. These results indicated that persistent activation of antilipolysis by SRIF increased the set-point of lipolysis by sensitizing (i.e., cross-regulating) lipolysis and by desensitizing antilipolysis.

(Key words: adipocyte, broiler, glucagon, lipolysis, somatostatin)

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INTRODUCTION

The hydrolysis of triglycerides in chicken adipocytes is regulated by hormones through the cyclic adenosine monophosphate (cAMP) signal transduction pathway. Glucagon stimulates, whereas pancreatic polypeptide (McCumbee and Hazelwood, 1977) and somatostatin (SRIF; Strosser et al., 1983) inhibit cAMP accumulation and lipolysis. Glucagon stimulates cAMP synthesis by binding to receptors on the cell-surface that are coupled to Gs, a G protein that activates adenylyl cyclase. It is not known whether pancreatic polypeptide lowers intracellular cAMP by inhibiting cAMP synthesis, by stimulating cAMP degradation through activation of phosphodiesterase, or both. Somatostatin does not lower intracellular cAMP by activating phosphodiesterase (Dorflinger and Schonbrunn, 1983; Strosser et al., 1983). Rather, SRIF reduces cAMP synthesis by binding to receptors on the cell-surface that are coupled to Gi, a G protein that inhibits adenylyl cyclase (Murray-Whelan and Schlegel, 1992; Luthin et al., 1993).

In addition to acute activation and deactivation of proteins that mediate lipolysis, hormones regulate triglyceride hydrolysis through chronic effects on expression of adipocyte proteins. Alterations in adipocyte protein expression are manifest as changes in the set-point of lipolysis, as changes in adipocyte responses to acute actions of hormones, or both. For example, chronic preincubation of broiler adipocytes with triiodothyronine increases the set-point of lipolysis and decreases maximal responsiveness of adipocytes to SRIF (Harden and Oscar, 1993; Suniga and Oscar, 1994).

Desensitization is a phenomenon whereby the response of a target cell to a continuous stimulus decreases over time (Perkins et al., 1991; Lohse, 1993). Desensitization is common for hormones that regulate adenylyl cyclase through receptors coupled to Gi proteins. The mechanism of desensitization involves both acute and chronic effects of hormones. Short-term desensitization involves uncoupling of signal transduction between the receptor and Gi protein due to phosphorylation of the receptor, whereas long-term desensitization involves down-regulation of hormone receptors and Gi proteins.

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Desensitization phenomena are involved in the regulation of lipolysis in chicken adipocytes. Glucagon induces desensitization of lipolysis stimulation (Campbell and Scanes, 1987; Oscar, 1992) and down-regulates its receptor on broiler adipocytes (Oscar, unpublished data). On the other hand, persistent inhibition of lipolysis by pancreatic polypeptide does not induce desensitization of antilipolysis in broiler adipocytes (Oscar, 1993).

The ability of SRIF to induce desensitization of antilipolysis in chicken adipocytes has not been investigated, but is suggested by studies with mammalian cells. In mouse anterior pituitary tumor cells, SRIF induces desensitization of its ability to acutely inhibit adenylyl cyclase (Reisine, 1984), and induces a rapid down-regulation of its receptors (Mahy et al., 1988). Likewise, in S49 lymphoma cells (Mayor et al., 1987) and CHO-K1 cells (Vanetti et al., 1993), SRIF induces desensitization of its ability to inhibit adenylyl cyclase. However, Presky and Schonbrunn (1988) reported that prolonged exposure of GH4C1 rat pituitary tumor cells to SRIF does not induce desensitization of adenylyl cyclase inhibition. Transfection experiments indicate that the ability of a target cell to undergo desensitization to SRIF depends on the subtype(s) of SRIF receptors expressed. Somatostatin receptor subtype 2B, a splice variant with a truncated C terminus, does not undergo desensitization and down-regulation when expressed in CHO-K1 cells (Vanetti et al., 1993). The complement of SRIF receptor subtypes in chicken adipocytes is unknown and, therefore, it is not possible to predict whether SRIF induces desensitization of antilipolysis.

In addition to desensitization, lipolysis in broiler adipocytes is affected by cross-regulation, a phenomenon whereby persistent activation of one pathway leads to sensitization of the counter-regulatory pathway (Hadcock et al., 1992). Persistent activation of antilipolysis by pancreatic polypeptide increases the set-point of lipolysis (Oscar, 1993). Whether SRIF cross-regulates lipolysis in a similar manner is not known.

The regulation of lipolysis in adipocytes is complex, and involves acute and chronic effects of hormones that are integrated in phenomena such as desensitization and cross-regulation to alter the set-point of lipolysis, as well as adipocyte responses to fast-acting lipolytic and antilipolytic hormones. In the present study, broiler adipocytes in primary culture were used to characterize acute and chronic effects of SRIF on lipolysis and antilipolysis, and to determine whether desensitization and cross-regulation phenomena are involved in the regulation of adipocyte metabolism by SRIF.

### MATERIALS AND METHODS

#### Broilers

Female broilers (Ross x Avian) were obtained weekly at 1 d of age from a local hatchery. Chicks were raised in a floor pen (1.5 x 1.25 m) equipped with two nipple waterers and a 10-kg capacity tube feeder, and covered with pine shavings. Supplemental heat and a satellite waterer were provided from 1 to 7 d of age. Meat Builder poultry feed and water were provided for ad libitum consumption under a 23 h light:1 h dark cycle. Birds were used as a source of adipose tissue between 42 and 49 d of age. They were killed rapidly (< 30 s) by carbon dioxide asphyxiation. Animal care procedures and euthanasia were approved and monitored by the USDA, Agricultural Research Service, Institutional Animal Care and Use Committee.

#### Isolation and Culture of Adipocytes

Adipocytes were isolated and cultured as previously described (Oscar, 1991; Harden and Oscar, 1993). Briefly, abdominal fat was removed and digested for 1 h at 37 °C under sterile conditions. The sterile digestion medium was a Dulbecco's modified Eagle's base medium supplemented with glucose (10 mM), pyruvate (1 mM), glutamine (1 mM), sodium bicarbonate (4 mM), HEPES (1 mM), phenol red (14 μM), BSA (3% wt/vol), collagenase (250 U/mL), and trypsin (0.125%) at pH 7.6. The digestion mixture was filtered through polypropylene mesh to remove intact tissue and then adipocytes were isolated from other cells by flotation and washing.

Isolated adipocytes (1% vol/vol) were cultured at 37 °C in 50-mL polystyrene centrifuge tubes that contained 30 mL of sterile incubation medium under an initial atmosphere of air. The sterile incubation medium was similar in composition to the sterile digestion medium except that it did not contain digestion enzymes, contained only 1% BSA, and was supplemented with antibiotics (50 units/mL of penicillin G, 50 μg/mL of streptomycin, and 100 μg/mL of neomycin).

#### Pretreatment of Adipocytes

Adipocytes cultured for 48 h were pretreated with 0 to 30 nM SRIF5 for 0 to 24 h before washing and assessment of lipolysis. Adipocytes were washed thrice with wash medium, which was similar in composition to sterile incubation medium except that it did not contain phenol red, pyruvate, or antibiotics. Twenty-minute incubations at 37 °C were included in the first two wash steps to facilitate removal of cell-associated SRIF.

#### Lipolysis Assay

Lipolysis assays involved incubating 3% vol/vol adipocytes for 1 h at 37 °C in 300 μL of assay medium that contained 0 to 2.7 nM mammalian glucagon (mixture of

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3Townsend, Millsboro, DE 19966.
4Purina Mills, Saint Louis, MO 63166.
5Somatostatin-14, Sigma Chemical Co., Saint Louis, MO 63178-9916.
TABLE 1. Effect of preincubation dose of somatostatin (SRIF) on mean diameter and number of broiler adipocytes used in lipolysis assays

<table>
<thead>
<tr>
<th>Adipocyte characteristic</th>
<th>0 nM</th>
<th>0.3 nM</th>
<th>3 nM</th>
<th>30 nM</th>
<th>SEM</th>
<th>p²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean diameter, μm</td>
<td>68.5</td>
<td>68.2</td>
<td>69.1</td>
<td>68.7</td>
<td>0.21</td>
<td>0.13</td>
</tr>
<tr>
<td>Number, per 3% vol/vol</td>
<td>39,616</td>
<td>40,238</td>
<td>40,336</td>
<td>40,616</td>
<td>1,070</td>
<td>0.93</td>
</tr>
</tbody>
</table>

¹Adipocytes were preincubated at 37°C for 24 h with the indicated doses of SRIF. Data are from 20 replicates in which adipocytes were prepared from a single donor bird in each replicate.

²Probability for no effect of preincubation dose of SRIF.

Adipocytes used in lipolysis assays were fixed overnight at 22°C in 2 mL of 2% osmium tetroxide in collidine buffer (Hirsch and Gallian, 1968). The fixed adipocytes were collected on a 15-μm nylon screen, washed with 60 mL of 0.9% NaCl, resuspended in 200 mL of 0.9% NaCl, and counted and sized using a Coulter Multisizer II.

RESULTS

The mean diameter of adipocytes used to assess lipolysis was not affected by chronic exposure to SRIF (Table 1). Likewise, the number of adipocytes used in the lipolysis assays was similar across preincubation doses of SRIF. Consequently, expression of glycerol release on an adipocyte volume (i.e., 3% vol/vol) basis, as done throughout this study, was equivalent to expression on an adipocyte number basis.

Preincubation of adipocytes with SRIF for 24 h induced a dose-response enhancement (P = 0.0001) of basal lipolysis and lipolysis stimulated by 0.08 or 0.27 nM glucagon (Figure 1). Maximal enhancement of lipolysis was observed at preincubation doses of 15 and 30 nM. Lipolysis in the presence of 2.7 nM glucagon was similar (P = 0.46) in control and pretreated adipocytes.

The effect of time of preincubation with SRIF on lipolysis in broiler adipocytes is shown in Figure 2. Lipolysis in control adipocytes was constant during the time course of this experiment. In contrast, preincubation of adipocytes with 30 nM SRIF enhanced (time by SRIF at P = 0.0002) basal lipolysis 14 nmol/h at 0.5 h, 18 nmol/h at 1 h, 23 nmol/h at 2 h, and 28 nmol/h at 3 h.

FIGURE 1. Effect of preincubation dose of somatostatin (SRIF) on lipolysis in broiler adipocytes. Adipocytes were pretreated with 0, 1.5, 3, 6, 15, and 30 nM SRIF for 24 h at 37°C. Lipolysis stimulated by 0 (○), 0.08 (□), 0.27 (▲), or 2.7 (●) nM glucagon was assessed during a subsequent 1 h incubation at 37°C. This experiment involved four replicates in which adipocytes were isolated from a single donor bird in each replicate. Each point is the mean ± SEM.
FIGURE 3. Effect of preincubation with somatostatin (SRIF) on glucagon stimulation of lipolysis in broiler adipocytes. Adipocytes were pretreated with 0 (○) or 30 (●) nM SRIF for 24 h at 37°C. Lipolysis was assessed during a subsequent 1 h incubation at 37°C with the indicated doses of glucagon. Net potentiation of glycerol release (inset) was calculated by subtracting glycerol release of control adipocytes from glycerol release of pretreated adipocytes. This experiment involved three replicates in which adipocytes were isolated from a single donor bird in each replicate. Each point or bar is the mean ± SEM.

nmol/h at 4 h, and 54 nmol/h at 24 h (Figure 2a). Lipolysis stimulated by 0.08 nM glucagon increased (time at P = 0.051; SRIF at P = 0.0001) 16 nmol/h at 0.5 h, 22 nmol/h at 4 h, and 36 nmol/h at 24 of pretreatment (Figure 2b). Regardless of time of preincubation, in the presence of 0.27 nM glucagon (Figure 2c), lipolysis was 20 nmol/h higher (SRIF at P = 0.0001) and in the presence of 2.7 nM glucagon (Figure 2d), lipolysis was 18 nmol/h higher (SRIF at P = 0.0001) in adipocytes pretreated with SRIF.

Analysis of full dose-response curves for glucagon stimulation of lipolysis revealed that pretreatment with SRIF potentiated (P = 0.0001) basal lipolysis and lipolysis stimulated by submaximal stimulatory doses of glucagon but did not alter lipolysis in response to maximal stimulatory doses of glucagon (Figure 3). The net potentiation of basal lipolysis (44 nmol/h) was greater than the net potentiation of submaximal lipolysis (0 to 34 nmol/h), indicating that adipocyte sensitivity to glucagon was not altered by SRIF pretreatment (Figure 3 inset).

Acute exposure of control adipocytes to SRIF resulted in a dose-response inhibition (P = 0.0001) of basal and glucagon-stimulated lipolysis (Figure 4). Basal lipolysis was reduced from 39 to 18 nmol/h, lipolysis stimulated by 0.08 nM glucagon was decreased from 82 to 21 nmol/h, lipolysis stimulated by 0.27 nM glucagon was reduced from 96 to 40 nmol/h, and lipolysis stimulated by 2.7 nM glucagon was decreased from 116 to 102 nmol/h. The ID50 for SRIF increased (P = 0.006) from 28 to 52 to 153 to 500 pM as the concentration of glucagon used to stimulate lipolysis increased from 0 to 0.08 to 0.27 to 2.7 nM. Thus, the potency and extent of lipolysis inhibition by SRIF was inversely related to the concentration of glucagon used to stimulate lipolysis.

In addition to acute stimulation of lipolysis by glucagon, pretreatment of adipocytes with SRIF reduced the ability of SRIF to inhibit lipolysis (Table 2); this was indicated by a rightward shift of the dose-response curves for SRIF (Figure 5). More specifically, in the absence of glucagon in the assay, adipocytes pretreated with SRIF exhibited enhanced glycerol release and reduced sensitivity but normal maximal responsiveness to SRIF. In the presence of 0.08 nM glucagon in the assay, preincubation of adipocytes with SRIF increased glycerol release and decreased sensitivity and maximal responsiveness to SRIF, whereas in the presence of 0.27 nM glucagon in the assay, SRIF-pretreated adipocytes displayed decreased sensitivity and maximal responsiveness to SRIF even though glycerol release was similar to that of control adipocytes. The latter result, attenuated antilipolysis in the absence of enhanced lipolysis, was consistent with SRIF-inducing desensitization of antilipolysis.

Pretreatment effects of SRIF on antilipolysis were dose-dependent (Table 3). The dose of SRIF causing half-maximal inhibition (ID50) of basal lipolysis was in-
FIGURE 2. Effect of time of preincubation with somatostatin (SRIF) on lipolysis in broiler adipocytes. Adipocytes were preincubated with 0 (○) or 30 (●) nM SRIF for 0.5, 4, or 24 h at 37 C and then lipolysis was assessed during an ensuing 1 h incubation at 37 C with a) 0, b) 0.08, c) 0.27, or d) 2.7 nM glucagon. This experiment involved six replicates in which adipocytes were isolated from a single donor bird in each replicate. Each point is the mean ± SEM.

TABLE 2. Effect of preincubation with somatostatin (SRIF) on SRIF inhibition of lipolysis in broiler adipocytes

<table>
<thead>
<tr>
<th>Glucagon (nM)</th>
<th>0 nM</th>
<th>30 nM</th>
<th>SEM</th>
<th>( P^4 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GR, nmol/h</td>
<td>ID_{50}, pM</td>
<td>MI, %</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>24</td>
<td>15</td>
<td>60</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>76</td>
<td>32</td>
<td>56</td>
<td>0.051</td>
</tr>
<tr>
<td>0.08</td>
<td>84</td>
<td>52</td>
<td>67</td>
<td>0.027</td>
</tr>
<tr>
<td></td>
<td>112</td>
<td>63</td>
<td>114</td>
<td>0.051</td>
</tr>
<tr>
<td>0.27</td>
<td>108</td>
<td>87</td>
<td>167</td>
<td>0.043</td>
</tr>
<tr>
<td></td>
<td>114</td>
<td>63</td>
<td>114</td>
<td>0.50</td>
</tr>
</tbody>
</table>

1Adipocytes were preincubated at 37 C with the indicated doses of SRIF for 24 h, washed, and then incubated at 37 C for 1 h with 0 to 3 nM SRIF in the presence of 0, 0.08, or 0.27 nM glucagon. This experiment involved four replicates in which adipocytes were isolated from a single donor bird in each replicate.

2Dose of glucagon used in the lipolysis assay.

3GR = glycerol release in the absence of SRIF; ID_{50} = dose of SRIF causing half-maximal inhibition of lipolysis; MI = maximal inhibition of lipolysis by SRIF.

4Probability for no effect of preincubation with SRIF.
increased 0.1-, 1.3-, and 2-fold by prolonged exposure of adipocytes to 0.3, 3, and 30 nM SRIF, respectively. Again, the ability of SRIF to maximally inhibit basal lipolysis was not altered by SRIF pretreatment. Although basal glycerol release from adipocytes pretreated with 0.3 nM SRIF was enhanced 27 nmol/h, the ability of SRIF to inhibit lipolysis was not attenuated. This finding, enhanced lipolysis in the absence of attenuated antilipolysis, indicated that SRIF sensitized (i.e., cross-regulated) lipolysis independent of its effects on antilipolysis.

The attenuation of antilipolysis occurred predominantly after 4 h of preincubation with SRIF and, therefore, represented a chronic effect of SRIF on antilipolysis (Table 4). The ID_{50} for SRIF inhibition of glucagon-stimulated lipolysis increased 0.3-fold at 0.5 h, 0.8-fold at 4 h, and 3.5-fold at 24 h of pretreatment. Maximal inhibition of lipolysis decreased 7% at 0.5 h, 11% at 4 h, and 62% at 24 h of preincubation. Thus, reductions in adipocyte sensitivity and maximal responsiveness to SRIF at 0.5 h were only 10% and at 4 h were only 20% of those observed at 24 h of preincubation. Although preincubation with SRIF induced a time-dependent attenuation of antilipolysis in this experiment, glycerol release from control and pretreated adipocytes were similar. Again, this result, attenuated antilipolysis in the absence of enhanced lipolysis, was consistent with SRIF inducing desensitization of antilipolysis.

The absolute response of adipocytes to acute stimulation of lipolysis by 80 pM glucagon was inconsistent between experiments, as shown in Figure 2b and Table 4. These experiments were conducted 7 mo apart, and, although experimental procedures were standardized, subtle changes in experimental conditions, such as use of different lots of enzymes, hormones, media, and birds, could have produced inconsistent absolute responses to glucagon by altering expression of adipocyte genes.

### Table 3. Effect of preincubation dose of somatostatin (SRIF) on SRIF inhibition of basal lipolysis in broiler adipocytes

<table>
<thead>
<tr>
<th>Preincubation dose of SRIF</th>
<th>0 nM</th>
<th>0.3 nM</th>
<th>3 nM</th>
<th>30 nM</th>
<th>SEM</th>
<th>p^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>GR, nmol/h</td>
<td>36</td>
<td>63</td>
<td>80</td>
<td>89</td>
<td>3</td>
<td>0.0001</td>
</tr>
<tr>
<td>ID_{50}, pM</td>
<td>21</td>
<td>24</td>
<td>49</td>
<td>64</td>
<td>6</td>
<td>0.0015</td>
</tr>
<tr>
<td>MI, %</td>
<td>54</td>
<td>60</td>
<td>62</td>
<td>62</td>
<td>2</td>
<td>0.15</td>
</tr>
</tbody>
</table>

^1 Adipocytes were preincubated at 37 C for 24 h with the indicated doses of SRIF, washed, and then incubated at 37 C for 1 h with 0 to 3 nM SRIF in the absence of glucagon. This experiment involved four replicates in which adipocytes were isolated from a single donor bird in each replicate.

^2 GR = glycerol release in the absence of SRIF; ID_{50} = dose of SRIF causing half-maximal inhibition of lipolysis; MI = maximal inhibition of lipolysis by SRIF.

^3 Probability for no effect of preincubation dose of SRIF.
cyte proteins that propagate (i.e., glucagon receptors, Gs, adenylyl cyclase) and terminate (i.e., Gs, phosphodiesterase, proteases) glucagon’s acute effects on lipolysis. Regardless, changes in adipocyte responses to glucagon between experiments did not compromise findings of this study because effects of SRIF on lipolysis and antilipolysis were evaluated within experiments using relative comparisons between control and pretreated adipocytes.

DISCUSSION

In addition to inhibition of cAMP synthesis, SRIF inhibits lipolysis in chicken adipocytes by a cAMP-independent mechanism. Strosser et al. (1983) reported that stimulation of lipolysis in chicken adipocytes by dibutyryl cAMP, a nondegradable analog of cAMP, is inhibited by SRIF. In the present study, when lipolysis was maximally stimulated by 2.7 nM glucagon, a situation in which cAMP should not limit lipolysis, SRIF reduced glycerol release from 116 to 102 nmol/h (Figure 4). Considering that basal lipolysis was reduced to 18 nmol/h in the same experiment, 14% of the inhibitory effect of SRIF on lipolysis can be attributed to an effect distal to cAMP.

Activation of serine phosphatases, such as protein phosphatase 1 or 2A, in adipocytes is a proposed mechanism whereby antilipolytic hormones exert cAMP-independent effects on lipolysis (Yeaman, 1990). Protein phosphatases deactivate hormone-sensitive lipase, the rate-limiting enzyme of triglyceride hydrolysis in adipocytes, by dephosphorylating serine 563, the regulatory phosphorylation site. Although proof that SRIF inhibits lipolysis by this mechanism is not available, in GH4C1 pituitary tumor cells SRIF stimulates K+ channels by a cAMP-independent mechanism that involves protein dephosphorylation (White et al., 1991).

In addition to acute effects, hormones have chronic effects on expression of proteins that mediate lipolysis in adipocytes. Alterations in expression of adipocyte proteins following chronic pretreatment with a hormone are manifest as changes in the set-point of lipolysis, as changes in adipocyte responses to acute actions of hormones, or both. In the current study, preincubation of adipocytes with SRIF produced a dose-response and time-dependent increase in the set-point of lipolysis and reduced adipocyte sensitivity and maximal responsiveness to SRIF while not altering sensitivity of adipocytes to glucagon. In comparison, prolonged exposure of broiler adipocytes to pancreatic polypeptide increases the set-point of lipolysis, reduces maximal responsiveness of adipocytes to SRIF and pancreatic polypeptide, but has no effect on adipocyte sensitivity to glucagon, SRIF, or pancreatic polypeptide (Oscar, 1993).

Although pretreatment of broiler adipocytes with pancreatic polypeptide (Oscar, 1993) or SRIF (the current study) markedly increases the set-point of lipolysis, the effect of these pretreatments on maximal lipolysis differ. Preincubation with 12 nM pancreatic polypeptide, a maximally effective dose, for 24 h increases basal lipolysis by 27 nmol/h and maximal lipolysis by 13 nmol/h (Oscar, 1993). In contrast, preincubation with 30 nM SRIF, a maximally effective dose, for 24 h did not alter maximal lipolysis but increased basal lipolysis by 54 nmol/h (Figure 1). Assuming that cAMP does not limit maximal lipolysis, these results suggest that 50% of the enhancement of lipolysis in adipocytes pretreated with pancreatic polypeptide is due to effects distal to cAMP formation, whereas 100% of the enhancement of lipolysis in adipocytes preincubated with SRIF was due to effects on cAMP formation.

In the dose-response experiment shown in Table 3, adipocytes pretreated with 0.3 nM SRIF exhibited enhanced basal lipolysis in the absence of attenuated antilipolysis; a finding consistent with cross-regulation of lipolysis by SRIF. On the other hand, there were two examples (Tables 2 and 4) in which attenuated antilipolysis was observed in the absence of enhanced lipolysis; observations consistent with SRIF inducing desensitization of antilipolysis. Taken together these results suggest that the increased set-point of lipolysis in adipocytes pretreated with SRIF was due to both sensitized (i.e., cross-regulated) lipolysis and desensitized antilipolysis; perhaps as a consequence of up-regulation of proteins.

### TABLE 4. Effect of time of preincubation with somatostatin (SRIF) on SRIF inhibition of glucagon-stimulated lipolysis in broiler adipocytes

<table>
<thead>
<tr>
<th>Time of preincubation</th>
<th>Item 2</th>
<th>0 h</th>
<th>0.5 h</th>
<th>4 h</th>
<th>24 h</th>
<th>SEM</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>GR, nmol/h</td>
<td></td>
<td>113</td>
<td>117</td>
<td>113</td>
<td>107</td>
<td>3</td>
<td>0.44</td>
</tr>
<tr>
<td>ID50, pM</td>
<td></td>
<td>111</td>
<td>141</td>
<td>205</td>
<td>497</td>
<td>35</td>
<td>0.0008</td>
</tr>
<tr>
<td>MI, %</td>
<td></td>
<td>71</td>
<td>66</td>
<td>63</td>
<td>27</td>
<td>3</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

1 Adipocytes were preincubated at 37 C for 30 nM SRIF for the indicated times, washed, and then incubated at 37 C for 1 h with 0 to 3 nM SRIF in the presence of .08 nM glucagon. This experiment involved three replicates in which adipocytes were isolated from a single donor bird in each replicate.

2 GR = glycerol release in the absence of SRIF; ID50 = dose of SRIF causing half-maximal inhibition of lipolysis; MI = maximal inhibition of lipolysis by SRIF.

3 Probability for no effect of time of preincubation with SRIF.
(i.e., $G_s$, adenylyl cyclase) that stimulate cAMP formation and down-regulation (i.e., SRIF receptors, $G_i$, phosphodiesterase) of proteins that inhibit cAMP accumulation. In contrast, the increased set-point of lipolysis in broiler adipocytes pretreated with pancreatic polypeptide is entirely attributable to sensitized lipolysis as preincubation with pancreatic polypeptide does not induce desensitization of antilipolysis (Oscar, 1993).

Persistent inhibition of lipolysis in mammalian adipocytes also sensitizes lipolysis and desensitizes antilipolysis (Hoffman et al., 1986; Green, 1987). These changes in adipocyte responses to acute actions of lipolytic and antilipolytic agents are associated with changes in expression of adipocyte proteins. Green et al. (1992) reported that prolonged exposure of rat adipocytes to phenylisopropyladenosine (PIA), an adenosine analog, reduces adenosine receptors by 60% and $G_i$ by 50 to 90%, whereas expression of $G_s$ and activity of adenylyl cyclase are unaltered. Similar changes in expression of adenosine receptors and $G_i$ are observed in adipocytes from rats infused with PIA for 6 d (Longabaugh et al., 1989). However, prolonged in vivo exposure to PIA increases $G_s$ by 40 to 50% (Longabaugh et al., 1989), whereas $\beta$-adrenergic receptor numbers are not altered (Parsons and Stiles, 1987).

In hamster smooth muscle cells, persistent activation of adenylyl cyclase inhibition with PIA enhances sensitivity and maximal responsiveness to isoproterenol, a $\beta$-adrenergic receptor agonist, and is associated with a 70% increase of $\beta$-adrenergic receptors, a 50% reduction of adenosine receptors, a 50% reduction of $G_i$, and no changes in $G_s$ (Haddock et al., 1992). No changes in $G_s$ or $G_i$ are noted when mouse anterior pituitary tumor cells are preincubated with SRIF (Mayh et al., 1988). However, desensitization of SRIF inhibition of adenylyl cyclase is associated with a rapid reduction in SRIF binding. Thus, in general, persistent activation of receptors coupled to inhibition of adenylyl cyclase via $G_i$ in mammalian cells causes a down-regulation of inhibitory receptors and $G_i$ with variable effects on $G_s$ and receptors that activate adenylyl cyclase. The molecular mechanisms responsible for sensitization of lipolysis and desensitization of antilipolysis in broiler adipocytes pretreated with SRIF have not been investigated but are likely to involve changes in expression of proteins that stimulate and inhibit cAMP formation.

In summary, pretreatment of broiler adipocytes with SRIF increased the set-point of lipolysis through a combination of sensitized lipolysis and desensitized antilipolysis. Approximately 80% of the changes in lipolysis and antilipolysis induced by SRIF occurred after 4 h of preincubation, suggesting that SRIF had chronic effects on expression of adipocyte proteins. Further studies are needed in which expression of adipocyte proteins are measured following pretreatment with SRIF. Such studies will help us to better understand the mechanism(s) whereby SRIF regulates lipolysis and antilipolysis in broiler adipocytes.

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