

Implications of feedback regulation of beta-adrenergic signaling¹

S. E. Mills²

Department of Animal Science, Purdue University, West Lafayette, IN 47907

ABSTRACT: Receptor-mediated signals are tightly regulated by feedback inhibition and act to prevent signal overload and to reset the receptor to a changing environment. Short-term regulation (uncoupling) of beta-adrenergic receptors (β AR) involves receptor phosphorylation and uncoupling of the receptor from the G protein Gs. Chronic exposure to ligand leads to reduced receptor number (down-regulation), which results from a combination of receptor internalization and degradation, and decreased mRNA abundance. The extent of β AR regulation is subtype-specific with a rank order of β_2 AR > β_1 AR > β_3 AR. Differences between species are expected also because amino acid sequences differ. Uncoupling and down-regulation of β AR in pig tissues has been demonstrated in vivo and in vitro, although skeletal muscle exhibits a blunted response compared with adipose tissue and changes in mRNA abundance have not been observed. Desensitization presents a challenge

clinically in the treatment of human disease and may well limit the effectiveness of β AR ligands used to promote livestock production. Pigs fed β AR ligands show a rapid response in growth and feed efficiency that tends to peak during the first 7 to 10 d but declines thereafter toward zero by approximately 6 wk. A similar pattern was reported in rats fed clenbuterol and was accompanied by a 50% reduction in β AR in skeletal muscle. Feeding clenbuterol every 2nd d prevented the decline in the response to clenbuterol and gave a growth response that was equivalent to daily dosing. These data suggest that strategies to prevent or circumvent β AR down-regulation may prolong the agonist response. Intermittent dosing of pigs may present logistical problems. An alternative approach may be to incrementally increase the dose of β AR ligand to compensate for the decline in response or to augment the ligand response by inhibiting the inhibitory G protein Gi.

Key Words: Beta-Adrenergic Receptor, Pigs, Regulation

©2002 American Society of Animal Science. All rights reserved.

J. Anim. Sci. 80(E. Suppl. 1):E30–E35

Introduction

Living organisms operate in a state of imbalance and the neural and endocrine systems have evolved to modify the rates of physiological processes to maintain homeostasis. Whether we have binged on chocolate-chip cookies, fasted for 10 d, or suddenly felt the need to run a marathon, these two systems interact to fine-tune metabolic rate. One of the hallmarks of both regulatory systems is the short-lived nature of the signals produced. The half-life of neurotransmitters is measured in seconds, whereas that of circulating hormones may be minutes to days. The rationale for the need for a system that is short-lived is to permit our systems to reset in order to meet the next challenge.

The short-lived nature of chemical messengers extends to receptor molecules and signaling responses. Readjustments in these systems are multifaceted and include uncoupling of receptor responses from signaling events, degradation of receptors, and up- and down-regulation of signaling molecules that affect the primary signaling pathway.

The need to regulate can be appreciated by considering several examples of disruption of regulation, including goiter, which results from overstimulation by thyroid-stimulating hormone when thyroid hormones are deficient (Hetzel, 1983), tumor growth due to mutations in signaling molecules (Sebolt-Leopold et al., 1999), and cholera, which results from overstimulation of G-protein signaling cascades (Fishman and Atikkan, 1980). Endocrine signals are tightly regulated so that animals can adapt to changing needs and to prevent the development of pathologies associated with unchecked signaling.

Beta-Adrenergic Receptors and Signaling

The beta-adrenergic (β AR) belong to a large family of seven transmembrane-domain proteins that couple

¹Journal paper no. 16655 of the Purdue Univ. Agric. Res. Prog. This paper was presented at the 2001 Midwest meetings of the Am. Soc. Anim. Sci. [79 (Suppl. 2):53 (Abstr.)].

²Correspondence: Lilly Hall (phone: 765-494-4845; fax: 765-494-9346; E-mail: smills@purdue.edu).

Received April 5, 2001.

Accepted October 30, 2001.

and signal through guanine nucleotide binding proteins (G-proteins). Three β AR subtypes have been cloned and all signal in a similar manner. The binding of agonist promotes the interaction between the intracellular domains of the β AR and the heterotrimeric G-protein Gs (Strosberg et al., 1993). This interaction catalyzes the exchange of GTP for GDP in the $G\alpha$ subunit and leads to the dissociation of $G\alpha$ from $G\beta\gamma$. The activated $G\alpha$ activates adenylyl cyclase, catalyzing the synthesis of cAMP from ATP. The cAMP in turn activates protein kinase A (PKA), leading to subsequent phosphorylation events. Activation of adenylyl cyclase is terminated by the hydrolysis of GTP by an intrinsic GTPase and $G\alpha$ recombines with $G\beta\gamma$. Opposing the stimulatory effect of Gs proteins on adenylyl cyclase is the inhibitory action of hormones that signal through Gi, including the α_2 -adrenergic and adenosine receptors.

The ability of β AR ligands to increase cAMP is measured in terms of potency (sensitivity) and efficacy (maximum response) and can be modified by altering any of the inputs affecting adenylyl cyclase activity. For instance, chronic treatment with a β AR antagonist increases the density of β AR in the plasma membrane and results in an increase in sensitivity (Glaubiger and Lefkowitz, 1977), whereas chronic treatment with a β AR agonist reduces the density of β AR and reduces sensitivity (Pecquery et al., 1984). The response to β AR can also be increased by ligands that signal through Gi (Cumbay and Watts, 2001). In this case, the increased response is not a result of increased density of β AR, but rather a component of G-protein signaling. In contrast, conditions in which Gi is elevated result in reduced efficacy of β AR ligands (Tepe and Liggett, 2000).

Desensitization of β AR Signaling

Desensitization is defined as the attenuation of response despite continued presence of the stimulus and has been demonstrated in a variety of systems. One early report from the Lefkowitz group demonstrated that frogs given four doses of isoproterenol over 24 h had greater than 50% reduced stimulation of erythrocyte adenylyl cyclase (Mukherjee et al., 1975). Similar changes were induced in adipocytes from hamsters given once-daily subcutaneous injections of epinephrine for 6 d (Pecquery et al., 1984). The primary alteration was a reduced maximal response to isoproterenol for the stimulation of glycerol release, cAMP accumulation, and adenylyl cyclase activity, with no appreciable change in sensitivity to isoproterenol. Reduced responsiveness of adipocytes has been observed also in rats with a norepinephrine-secreting tumor (Prokocimer et al., 1988). However, in this instance reduced sensitivity to isoproterenol was more pronounced than was reduced maximal response. The effects of chronic elevation of catecholamines shown for adipocytes and erythrocytes appear to be a general phenomenon and likely extend to most, if not all, tissues that express β AR.

The β AR ligand-mediated desensitization has been demonstrated in several in vitro systems, including ventricular strips (Temma et al., 1985), adipocytes (Mills and Orcutt, 1989; Ding et al., 2000), and a variety of cell lines (Su et al., 1980). The primary alteration is at the level of the β AR itself or β AR-Gs coupling, because adenylyl cyclase activity assessed by direct activation or via Gs is not impaired under conditions in which signaling through the β AR is reduced (Mukherjee et al., 1975; Hausdorff et al., 1990). Increased inhibitory activity of Gi was ruled out in studies in which Gi was inactivated with pertussis toxin, but recent studies have implicated some role of Gi in contributing to the reduced signaling through adenylyl cyclase (Tepe and Liggett, 2000).

Mechanisms of Agonist-Induced Desensitization

Multiple mechanisms contribute to desensitization and can be divided into acute "uncoupling" responses and chronic "down-regulation" responses (Figure 1). Uncoupling of the β AR from Gs prevents signaling to adenylyl cyclase and represents the post-stimulation sequence that prepares the β AR for re-stimulation. Uncoupling is initiated within seconds to minutes of agonist exposure and the extent of uncoupling is closely related to the potency of the ligand to stimulate adenylyl cyclase (Su et al., 1979; Su et al., 1980). Desensitization by partial agonists is directly related to their ability to activate adenylyl cyclase, whereas antagonists do not induce uncoupling (Su et al., 1980). Uncoupling is further characterized as being quickly and completely reversible upon agonist removal, does not require the synthesis of new protein, and does not involve a change in receptor density (Su et al., 1980; Hausdorff et al., 1990).

The primary mechanism for uncoupling involves phosphorylation of the β AR, which interferes with coupling to Gs. In addition, the uncoupled β AR has a reduced affinity for agonists that decreases the signaling response at submaximal ligand concentrations. Two kinases are involved in β AR phosphorylation. The first is a true "feedback" regulation by PKA, which is activated by cAMP from adenylyl cyclase (Hausdorff et al., 1990). Phosphorylation by PKA alters the conformation of β AR and its affinity for Gs. Because cAMP is a second messenger for a number of hormones, regulation by PKA can phosphorylate and uncouple the receptor involved in initiating the signal (homologous regulation) or other G protein-coupled receptors (heterologous regulation) (Richelsen and Pedersen, 1985; Hausdorff et al., 1990). A second family of kinases, the G-protein coupled receptor kinases (GRK), includes soluble enzymes that mediate only homologous desensitization. The specific enzyme that phosphorylates Ser and Thr residues in the carboxy tail of the β AR is β ARK1 (β -adrenergic receptor kinase 1). The β ARK1 enzyme phosphorylates only agonist-occupied β AR, or that con-

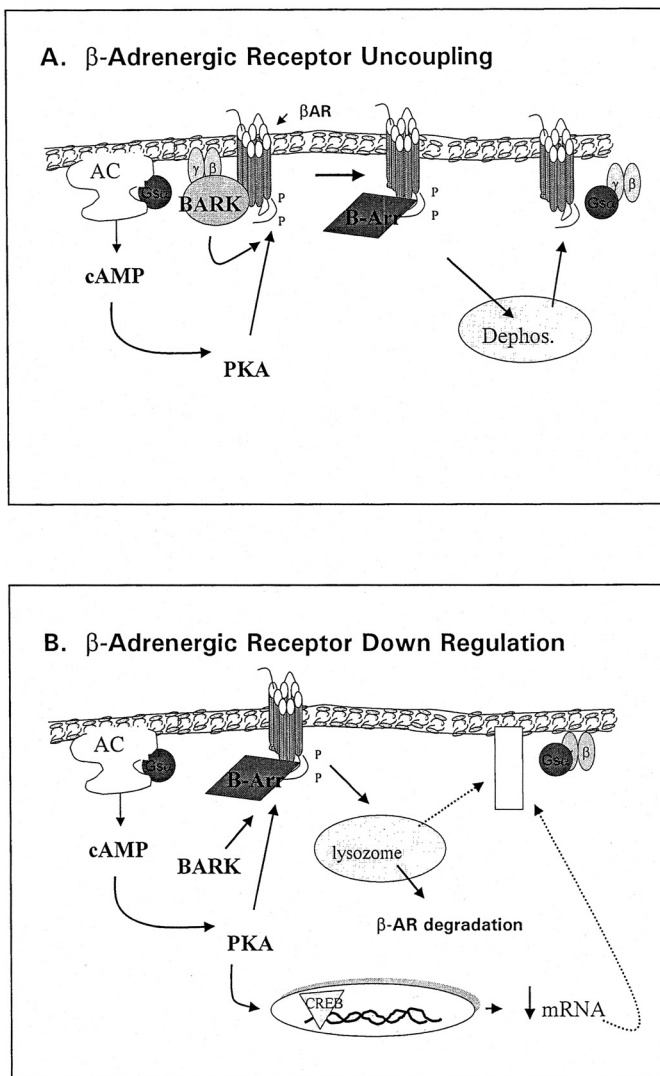


Figure 1. Cellular signals involved in β -adrenergic receptor uncoupling and down-regulation. Initial steps in desensitization are similar for uncoupling and down-regulation. Agonist binding leads to phosphorylation of the β AR by the kinases PKA and BARK I (A). The β AR subsequently binds β -arrestin (β AR-Arr), which effectively uncouples the receptor from further G-protein activation ($G_s\alpha$). The β AR is reactivated and recycled to the plasma membrane following dephosphorylation in intracellular vesicles. (B) Down-regulation differs from uncoupling in that internalized β AR are degraded in lysosomes and mRNA abundance for β AR may be decreased. Both processes contribute to a reduction in total β AR in the plasma membrane.

formation of the β AR induced by agonist binding (Liggett and Lefkowitz, 1994).

The sequence of events that results in β AR phosphorylation has been determined (Liggett and Lefkowitz, 1994). Upon ligand binding and G_s activation, $G_s\alpha$ associates with adenylyl cyclase, leaving $G_s(\beta\gamma)$ tethered to the membrane in close proximity to the β AR. The β ARK1 enzyme binds $G_s(\beta\gamma)$, effectively targeting the

kinase to the β AR. The agonist-occupied receptor has a high affinity for β ARK1, resulting in phosphorylation of multiple residues. Phosphorylation by β ARK1 increases the affinity of the β AR for one of the arrestin family of proteins (β -arrestin). The binding of β -arrestin causes steric hindrance of β AR association with $G_s\alpha$ and interrupts signaling. Receptors are subsequently reactivated by a process that involves sequestration and internalization into cytoplasmic vesicles, dephosphorylation, and recycling to the plasma membrane (Jockers et al., 1996; Kallal et al., 1998). Desensitization may also involve activation of G_i and inhibition of adenylyl cyclase (Tepe and Liggett, 2000). It has been shown that PKA phosphorylation of β AR increases the affinity for G_i , thus effectively switching signaling pathways from G_s to G_i .

Down-regulation is defined as a decline in the total number of β AR and contributes to desensitization from chronic exposure to agonists. Down-regulation develops more slowly than uncoupling, taking hours to days in cell culture systems (Su et al., 1980; Hausdorff et al., 1990) or in vivo (Mukherjee et al., 1975; Pecquery et al., 1984). The rate of decline in β AR binding sites is related to potency and efficacy of the drug (Su et al., 1980; Hausdorff et al., 1990). High concentrations of full agonists cause a more rapid decline in β AR binding, but even low concentrations of full agonists and partial agonists lead to loss of β AR number that is equivalent to that induced by a full agonist. Antagonists do not cause down-regulation, and in fact they have been shown to induce a compensatory increase in β AR number and responsiveness (Glaubiger and Lefkowitz, 1977).

Recovery from down-regulation is also slower than that from uncoupling, taking days in cell culture (Su et al., 1980). The total β AR protein content of a cell is the sum of the rates of synthesis and degradation, and it seems that both processes may contribute to the decline. Degradation of β AR involves endocytosis and hydrolysis in lysosomes. The process of endocytosis requires β ARK1 and β -arrestin, the latter of which binds to caltharin in coated pit regions of the plasma membrane and initiates endocytosis (Lefkowitz, 1998). If β ARK1/ β -arrestin are involved in both uncoupling and down-regulation, it is not clear whether these processes are always linked or a switch is made to induce down-regulation. It now appears that endocytosis is far more complex than simply β AR degradation and includes β AR reactivation and the activation of alternative signaling cascades (Lefkowitz, 1998).

Down-regulation of β AR occurs in cell lines expressing β AR genes that lack promoter elements, indicating that changes in expression are not required for down-regulation. Nonetheless, chronic exposure to β AR ligands leads to a decrease in mRNA abundance for the β_1 AR and β_2 AR (reviewed by Liggett and Lefkowitz, 1994) and likely contributes to the full decline in β AR protein.

Subtype Differences

Phosphorylation is a key element of desensitization, so it is logical that subtype difference in phosphorylation may determine rates and extent of desensitization. For the human β AR, the β_2 AR contains two potential PKA sites and 11 β ARK sites, whereas the β_1 AR has only one PKA site and 10 β ARK sites. The β_3 AR is the most divergent, having no PKA sites and only three β ARK sites (Hausdorff et al., 1990). Small differences have been noted between the β_1 AR and β_2 AR (Zhou et al., 1995), but the β_3 AR is resistant to short-term uncoupling responses (Granneman, 1992; Liggett and Lefkowitz, 1994). Chronic down-regulation of the β_3 AR may not involve the mechanisms outlined above because it appears to be absent in some cell types (Nantel et al., 1993; Liggett and Lefkowitz, 1994) but has been shown to develop slowly in others (Nantel et al., 1993). The pig β AR have been cloned and the putative phosphorylation sites are similar to those found in human β AR (Liang et al., 1997; Cao et al., 1998; Smith et al., 2000). The p β_1 AR has one potential PKA site and 10 Ser + Thr residues in the carboxy tail for β ARK phosphorylation. The p β_2 AR may have only one PKA site and 14 Ser + Thr residues. We would predict, therefore, that desensitization would be similar in the pig as described for other species. The p β_3 AR is similarly deficient in potential phosphorylation sites and its regulation would be expected to differ from the β_1 AR and β_2 AR.

Desensitization of Pig Tissues

Clenbuterol and cimaterol are two β AR agonists that induce muscle hypertrophy in rodents and are accompanied by a down-regulation of β AR in muscle tissue (Rothwell et al., 1987; Kim et al., 1992). Cimaterol similarly induced β AR down-regulation in pig skeletal muscle (Smith, 1989). Chronic feeding of ractopamine to swine reduced β AR in adipose tissue 25% after 1 d and up to 50% by 7 d (Spurlock et al., 1994). Curiously, however, significant β AR down-regulation was not detected in skeletal muscle and only trends for a decrease were observed. Smith (1989) also did not detect down-regulation of β AR in skeletal muscle of pigs fed ractopamine. Why do the adipose and muscle tissues appear to differ? One possibility is that β AR subtypes are distributed and regulated differently or that ractopamine preferentially targets one subtype. Indeed, the β_1 AR accounts for 75% of the β AR in adipose tissue and only 50% in skeletal muscle (Liang and Mills, 1999; McNeel and Mersmann, 1999). It is unknown whether these subtypes respond differently to elevated ligands, but ractopamine does not show specificity for one subtype over the other. One other possibility is that ractopamine might stimulate the synthesis of new β AR protein in skeletal muscle and effectively mask down-regulation. In adipose tissue, the decrease in β AR is consistent with the observed 40% decrease in rates of lipolysis and

a twofold decrease in sensitivity to ractopamine (Mills et al., 1990). In vitro incubation of pig adipocytes with isoproterenol reduced total β AR binding sites 40% but did not affect mRNA abundance for either the β_1 AR or β_2 AR (Ding et al., 2000).

Implications of β AR Desensitization

There is little question that desensitization can affect physiological processes and therapeutic approaches to the treatment of disease. For instance, a decrease in β AR density and responsiveness to catecholamines in heart muscle may precipitate heart failure (Choi and Rockman, 1999). Furthermore, β AR agonists are often administered to counteract desensitization in heart muscle, but the treatment may instead hasten desensitization and the loss of effectiveness of administered drugs (Colucci et al., 1981).

Animals treated with β AR agonists exhibit growth patterns indicative of desensitization. Weight gain in rats fed clenbuterol was increased 20% over the first 7 d, but the response diminished to zero by d 14. A similar pattern for weight gain was shown for pigs fed ractopamine (Williams et al., 1994) or L-644,969 (Convey et al., 1987). Maximal responses were observed in the first week to 10 d followed by a decline in the response to each drug to near zero by wk 7. Further, we favor the idea that down-regulation of β AR in adipose tissue prevents full expression of the β AR response and may lead to little or no change in the rate of fat accretion (Dunshie et al., 1993; Liu et al., 1994). If desensitization limits the effectiveness of β AR ligands, it is doubtful that effects are fully lost because removal of the ligands from the diet invariably results in a reversal of the favorable change in body composition (Jones et al., 1985; Mills and Orcutt, 1989).

Circumventing Desensitization

The question of greatest interest is, does desensitization limit the effectiveness of β AR agonists to modify growth and composition, and how can desensitization be prevented or overcome? One approach tested in rats was to feed clenbuterol every 2nd d, thereby allowing the β AR system an opportunity to reset (McElligot et al., 1989). Intermittent feeding seemed to prevent desensitization because growth rate was stimulated by clenbuterol to a similar extent at each supplement interval over 14 d, whereas rats fed clenbuterol continuously showed no response by d 14. Overall, growth rate and skeletal muscle gain were the same in the two groups, but the intermittently-fed rats received 50% less clenbuterol. It is not known whether intermittent feeding is advantageous over longer time periods. A second approach may be to incrementally increase the dose of drug to compensate for the decline in response. Drs. Schinckel and Richert at Purdue are taking this approach to determine whether a greater response can be achieved with ractopamine.

Implications

Compensatory desensitization is a well-characterized response to chronically elevated agonist concentration that results in a net loss of beta-adrenergic receptors (β AR) from the plasma membrane and reduced tissue response. The ability of β AR agonists to affect pig growth and alter body composition may well be limited by β AR desensitization. Experimental approaches should be investigated to determine the extent to which desensitization limits drug response, and to develop practical approaches to optimize the effectiveness of exogenously administered compounds. Three β AR subtypes have been cloned from mammalian species and most tissues express more than one subtype. It is not known which β AR subtypes are linked to growth responses in the pig or how β AR agonists affect the balance of synthesis and degradation of each subtype in different tissues. Answers to these questions may lead to targeting strategies that improve the efficacy of β AR ligands.

Literature Cited

- Cao, H., C. A. Bidwell, S. K. Williams, W. Liang, and S. E. Mills. 1998. Rapid communication: Nucleotide sequence of the coding region for the porcine beta 1-adrenergic receptor gene. *J. Anim. Sci.* 76:1720–1721.
- Choi, D. J., and H. A. Rockman. 1999. Beta-adrenergic receptor desensitization in cardiac hypertrophy and heart failure. *Cell Biochem. Biophys.* 31:321–329.
- Colucci, W. S., R. W. Alexander, G. H. Williams, R. E. Rude, B. L. Holman, M. A. Konstam, J. Wynne, G. H. Mudge, Jr., and E. Braunwald. 1981. Decreased lymphocyte beta-adrenergic-receptor density in patients with heart failure and tolerance to the beta-adrenergic agonist pirbuterol. *N. Engl. J. Med.* 305:185–190.
- Convey, E. M., E. Rickes, Y. T. Yang, M. A. McElligott, and G. Olson. 1987. Effects of beta-adrenergic agonist L-644,969 on growth performance, carcass merit and meat quality. In: *Proc. Recip. Meat Conf.*, St. Paul, MN. 40:47–55.
- Cumbay, M. G., and V. J. Watts. 2001. Heterologous sensitization of recombinant adenylyl cyclases by activation of D(2) dopamine receptors. *J. Pharmacol. Exp. Ther.* 297:1201–1209.
- Ding, S. T., E. O. Smith, R. L. McNeel, and H. J. Mersmann. 2000. Modulation of porcine adipocyte beta-adrenergic receptors by hormones and butyrate. *J. Anim. Sci.* 78:927–933.
- Dunshiea, F. R., R. H. King, R. G. Campbell, R. D. Sainz, and Y. S. Kim. 1993. Interrelationships between sex and ractopamine on protein and lipid deposition in rapidly growing pigs. *J. Anim. Sci.* 71:2919–2930.
- Fishman, P. H., and E. E. Atikkan. 1980. Mechanism of action of cholera toxin: effect of receptor density and multivalent binding on activation of adenylyl cyclase. *J. Membr. Biol.* 54:51–60.
- Glaubiger, G., and R. J. Lefkowitz. 1977. Elevated beta-adrenergic receptor number after chronic propranolol treatment. *Biochem. Biophys. Res. Commun.* 78:720–725.
- Granneman, J. G. 1992. Effects of agonist exposure on the coupling of beta 1 and beta 3 adrenergic receptors to adenylyl cyclase in isolated adipocytes. *J. Pharmacol. Exp. Ther.* 261:638–642.
- Hausdorff, W. P., M. G. Caron, and R. J. Lefkowitz. 1990. Turning off the signal: Desensitization of beta-adrenergic receptor function. *FASEB J.* 4:2881–2889.
- Hetzl, B. S. 1983. Iodine deficiency disorders (IDD) and their eradication. *Lancet* 2:1126–1129.
- Jockers, R., A. Da Silva, A. D. Strosberg, M. Bouvier, and S. Marullo. 1996. New molecular and structural determinants involved in beta 2-adrenergic receptor desensitization and sequestration. Delineation using chimeric beta 3/beta 2-adrenergic receptors. *J. Biol. Chem.* 271:9355–9362.
- Jones, R. W., R. A. Easter, F. K. McKeith, R. H. Dalrymple, H. M. Maddock, and P. J. Bechtel. 1985. Effect of the beta-adrenergic agonist cimaterol (CL 263,780) on the growth and carcass characteristics of finishing swine. *J. Anim. Sci.* 61:905–913.
- Kallal, L., A. W. Gagnon, R. B. Penn, and J. L. Benovic. 1998. Visualization of agonist-induced sequestration and down-regulation of a green fluorescent protein-tagged beta2-adrenergic receptor. *J. Biol. Chem.* 273:322–328.
- Kim, Y. S., R. D. Sainz, R. J. Summers, and P. Molenaar. 1992. Cimaterol reduces beta-adrenergic receptor density in rat skeletal muscles. *J. Anim. Sci.* 70:115–122.
- Lefkowitz, R. J. 1998. G protein-coupled receptors. III. New roles for receptor kinases and beta-arrestins in receptor signaling and desensitization. *J. Biol. Chem.* 273:18677–18680.
- Liang, W., C. A. Bidwell, S. K. Williams, and S. E. Mills. 1997. Rapid communication: Molecular cloning of the porcine β 2-adrenergic receptor gene. *J. Anim. Sci.* 75:2824.
- Liang, W., and S. E. Mills. 1999. Distribution of beta-adrenergic receptor subtypes in pig tissues. *FASEB J.* 13:A258.
- Liggett, S. B., and R. J. Lefkowitz. 1994. Adrenergic receptor-coupled adenylyl cyclase systems: Regulation of receptor function by phosphorylation, sequestration, and down-regulation. In: D. R. Sibley and M. D. Houslay (ed.) *Regulation of Cellular Signal Transduction Pathways by Desensitization and Amplification*. pp 71–96. John Wiley and Sons, New York.
- Liu, C. Y., A. L. Grant, K. H. Kim, S. Q. Ji, D. L. Hancock, D. B. Anderson, and S. E. Mills. 1994. Limitations of ractopamine to affect adipose tissue metabolism in swine. *J. Anim. Sci.* 72:62–67.
- McElligott, M. A., A. Barreto, Jr., and L. Y. Chaung. 1989. Effect of continuous and intermittent clenbuterol feeding on rat growth rate and muscle. *Comp. Biochem. Physiol.* 92C:135–138.
- McNeel, R. L., and H. J. Mersmann. 1999. Distribution and quantification of beta1-, beta2-, and beta3-adrenergic receptor subtype transcripts in porcine tissues. *J. Anim. Sci.* 77:611–621.
- Mills, S. E., C. Y. Liu, Y. Gu, and A. P. Schinckel. 1990. Effects of ractopamine on adipose tissue metabolism and insulin binding in finishing hogs. Interaction with genotype and slaughter weight. *Domest. Anim. Endocrinol.* 7:251–263.
- Mills, S. E., and A. L. Orcutt. 1989. Clenbuterol-induced desensitization in murine adipocytes: relationship to in vivo effectiveness. *Domest. Anim. Endocrinol.* 6:51–58.
- Mukherjee, C., M. G. Caron, and R. J. Lefkowitz. 1975. Catecholamine-induced subsensitivity of adenylyl cyclase associated with loss of beta-adrenergic receptor binding sites. *Proc. Natl. Acad. Sci. USA* 72:1945–1949.
- Nantel, F., H. Bonin, L. J. Emorine, V. Zilberfarb, A. D. Strosberg, M. Bouvier, and S. Marullo. 1993. The human beta 3-adrenergic receptor is resistant to short term agonist-promoted desensitization. *Mol. Pharmacol.* 43:548–555.
- Pecquery, R., M. C. Leneveu, and Y. Giudicelli. 1984. In vivo desensitization of the beta, but not the alpha 2-adrenoreceptor-coupled adenylyl cyclase system in hamster white adipocytes after administration of epinephrine. *Endocrinology* 114:1576–1583.
- Prokocimer, P. G., M. Maze, R. G. Vickery, and B. B. Hoffman. 1988. Mechanism for desensitization of beta-adrenergic receptor-stimulated lipolysis in adipocytes from rats harboring pheochromocytoma. *Endocrinology* 123:528–533.
- Richelsen, B., and O. Pedersen. 1985. Beta-adrenergic regulation of prostaglandin E2 receptors in human and rat adipocytes. *Endocrinology* 116:1182–1188.
- Rothwell, N. J., M. J. Stock, and D. K. Sudera. 1987. Changes in tissue blood flow and beta-receptor density of skeletal muscle in rats treated with the beta2-adrenoceptor agonist clenbuterol. *Br. J. Pharmacol.* 90:601–607.
- Sebolt-Leopold, J. S., D. T. Dudley, R. Herrera, K. Van Becelaere, A. Wiland, R. C. Gowan, H. Tecle, S. D. Barrett, A. Bridges, S. Przybranowski, W. R. Leopold, and A. R. Saltiel. 1999. Blockade

- of the MAP kinase pathway suppresses growth of colon tumors in vivo. *Nat. Med.* 5:810–816.
- Smith, C. K. 1989. Affinity of phenethanolamines for skeletal muscle beta-adrenergic receptors and influence on receptor down-regulation. *J. Anim. Sci.* 67(Suppl. 1):190 (Abstr.).
- Smith, T. R., C. A. Bidwell, and S. E. Mills. 2000. Rapid communication: Nucleotide sequence of the porcine β_3 -adrenergic receptor. *J. Anim. Sci.* 79:781–782.
- Spurlock, M. E., J. C. Cusamano, S. Q. Ji, D. B. Anderson, C. K. Smith, II, D. L. Hancock, and S. E. Mills. 1994. The effect of ractopamine on beta-adrenoceptor density and affinity in porcine adipose and skeletal muscle tissue. *J. Anim. Sci.* 72:75–80.
- Strosberg, A. D., L. Camoin, N. Blin, and B. Maigret. 1993. In receptors coupled to GTP-binding proteins, ligand binding and G-protein activation is a multistep dynamic process. *Drug Des. Discov.* 9:199–211.
- Su, Y. F., T. K. Harden, and J. P. Perkins. 1979. Isoproterenol-induced desensitization of adenylate cyclase in human astrocytoma cells. Relation of loss of hormonal responsiveness and decrement in beta-adrenergic receptors. *J. Biol. Chem.* 254:38–41.
- Su, Y. F., T. K. Harden, and J. P. Perkins. 1980. Catecholamine-specific desensitization of adenylate cyclase. Evidence for a multistep process. *J. Biol. Chem.* 255:7410–7419.
- Temma, K., T. Hirata, T. Kitazawa, and H. Kondo. 1985. Isoproterenol-induced desensitization to the positive inotropic effect of isoproterenol in ventricular strips isolated from carp heart (*Cyprinus carpio*). *Comp. Biochem. Physiol.* 82C:403–408.
- Tepe, N. M., and S. B. Liggett. 2000. Functional receptor coupling to Gi is a mechanism of agonist-promoted desensitization of the beta2- adrenergic receptor. *J. Recept. Signal Transduct. Res.* 20:75–85.
- Williams, N. H., T. R. Cline, A. P. Schinckel, and D. J. Jones. 1994. The impact of ractopamine, energy intake, and dietary fat on finisher pig growth performance and carcass merit. *J. Anim. Sci.* 72:3152–3162.
- Zhou, X. M., M. Pak, Z. Wang, and P. H. Fishman. 1995. Differences in desensitization between human beta 1- and beta 2-adrenergic receptors stably expressed in transfected hamster cells. *Cell. Signal.* 7:207–217.