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REVIEW ARTICLE

Multiple actions of β-adrenergic agonists on skeletal muscle and adipose tissue

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INTRODUCTION

β-Adrenergic agonists (β-agonists) are potent growth promoters in many species of animals [1–5]. This class of compounds produces a dramatic increase in skeletal muscle mass [1–3,6–9] and a large reduction in body fat content [2–4]. These findings have very important implications in several areas of human health, in addition to the clear impact of this drug in the animal meat producing industry.

Progress has been made to determine the mechanism of action of β-agonists. The prerequisite work to catalogue the effects of β-agonists has been documented in agricultural animal studies. At the functional level, β-agonists have profound influences on energy, carbohydrate, lipid and protein metabolism. At the tissue level, β-adrenergic receptors are present in all organs which are closely associated with growth such as skeletal muscle, adipose tissue and some neuro-endocrine organs. Although β-agonist actions on these physiological parameters are interesting phenomena, the biochemical aspect of the effects has not been thoroughly pursued. Detailed analysis is needed to elaborate the mode of action of β-agonists on muscle protein and fat metabolism. The physiological findings detailed below may provide the initial basis for investigations to understand these processes better.

SKELETAL MUSCLE HYPERTROPHY EFFECTS

Anabolic response

β-Agonists are the most potent agents that can promote normal skeletal muscle growth. A 10–20% increase in muscle weight is observed after treating rats with the β-agonist clenbuterol for only 1–2 weeks [2,7–9]. Lambs fed cimaterol for approximately 2 months showed a 25–30% increase in the weights of several muscles compared to lambs fed a control diet [3,6]. The gastrocnemius muscle in similarly treated lambs was reported to increase as much as 40% in weight [10].

The muscle growth in response to β-agonist treatment appears to be a true muscle hypertrophy, in contrast with other types of muscle growth, i.e. compensatory hypertrophy, in which satellite cell division precedes protein accumulation [11]. The muscle DNA concentration decreased in cimaterol-treated lambs [6,10] but the total DNA content was not altered. These results, coupled with the rapid growth response, which is observable within 2 days [7], suggests that satellite cell multiplication does not precede growth.

β-Agonist-induced hypertrophy is specific to striated muscle. Cardiac muscle size increases in response to β-agonists in some studies [1,9], but not in others in which skeletal muscle greatly increased in size [2]. Thus, the hypertrophy in heart may be different from that in skeletal muscle. The smooth muscle of the gut [9], the liver and kidney [2,9] do not increase in size in response to the anabolic action of these agents. This suggests that the controlling mechanisms of protein turnover in striated muscle tissue may be distinct from that in other tissues.

Time course

Time course analysis, using body weight gain as a non-invasive measure of muscle growth [7], demonstrated that the onset of the anabolic effect is rapidly observed within 2 days after feeding clenbuterol to rats and reaches a maximum within 8 days. The response attenuates after 14 days of treatment, and daily gain is the same as control. However, the previous increment of gain is retained. The early onset and later attenuation were also observed by direct measurement of muscle size [9]. Intermittent β-agonist feeding with a 2 day on-and-off regimen prevents attenuation [7]. The nature of this attenuation is not known, but is observed in several other models which measured different endpoints affected by β-agonist treatment [12,13]. The effect may be due to down-regulation of the β-agonist receptors. Rothwell *et al.* [14] showed that after chronic treatment with clenbuterol for 18 days there was a 50% reduction in β-receptor density in muscle.

Fibre type specificity

Although anabolic responses occur in both the soleus, a classical slow-twitch muscle, and the extensor digitorum longus (EDL), a classical fast-twitch muscle [7–9], histochemical observations suggest that the anabolic effect may be specific to certain fibre types. Muscle is composed of various ratios of Type I (slow-contracting, oxidative) and Type II (fast-contracting, mixed glycolytic/oxidative) fibres. β-Agonist treatment is consistently reported to increase the cross-sectional area of the Type II fibres (10–50%) in both rats [15,16] and lambs [6,10,17]. However, there is conflicting evidence concerning whether Type I fibres and fibre type composition are affected. Maltin *et al.* [15] reported an increase in the cross-sectional area of the slow oxidative fibres in the soleii of rats fed clenbuterol but no change in fibre type composition. The response was more apparent in rats fed the drug for 4 days than 21 days. There was no increase in cross-sectional area of any fibre type in the EDL but

Abbreviation used: EDL, extensor digitorum longus.

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a decrease in fast-twitch oxidative/glycolytic and an increase in fast-twitch glycolytic fibres. Zeman *et al.* [16] reported a hypertrophy in the soleus of the histochemically identified fast-twitch, but not slow-twitch, muscles in rats fed clenbuterol for 8–12 weeks and an increased ratio of fast- to slow-twitch. They also observed increased cross-sectional area of both fibre types in the EDL. Hypertrophy (10–20%) of Type I fibres and a decline in the percentage of Type I fibres were reported in muscle from lambs fed cimaterol for 7 and 12 weeks [6]. However, Kim *et al.* [10] found no hypertrophy in Type I fibres and no change in proportion of fibre types in lambs similarly treated for 8 weeks [10].

The compositional changes observed in some studies [6,16], with respect to fibre type switching from slow to fast is an interesting phenomena but appears to be associated with very long term β-agonist treatment. Fibre type switching may not be a primary event in protein accretion since the anabolic response attenuated [7] by the time the effect was apparent. Thus, the implications of this finding with regard to the mechanism of β-agonist action remains to be determined.

β-Receptor subtype

The β-receptors in various tissues have been classified into two subtypes, β_1 (cardiac contraction) and β_2 (bronchial smooth muscle relaxation), based on their potency ranking with adrenaline, noradrenaline and isoproterenol as originally proposed by Lands *et al.* [18]. More recently, β-agonists and antagonists with increasing selectivity for cardiac or tracheal receptors have been discovered. These highly 'selective' compounds were in turn used to classify tissue receptors or to determine the selectivity of other compounds using biological or receptor binding assays. This method of receptor classification was useful for drug discovery in the past, but may not be adequate now for a detailed inquiry into β-adrenergic biology. New technical advances will certainly add a needed dimension to β-adrenergic receptor classification in the future. Already, the β_2 receptor [19,20] and the β_1 receptor [21] have been cloned and their structure determined.

The β-adrenergic receptor subtype has been identified in muscle and is demonstrated to be predominantly β_2 [22–25]. Clenbuterol is one of the most potent of the growth-promoting β-agonists. The compound is highly active in biological assays on β_2 receptor type tissues but has little activity on β_1 subtype tissue [26], suggesting that the β_2 receptor subtype is involved in the muscle growth response. Interestingly, it was reported that propranolol did not inhibit clenbuterol-stimulated protein accretion but reduced the increase in muscle fibre size [27]. The significance of these results remains to be established.

Post-receptor events

In the absence of any solid contradictory evidence, the signal transduction sequence for β-adrenergic agonist action in muscle is presumed to be similar to that in other tissues. It is clear that β-agonists bind to β-receptors, stimulate adenylate cyclase activity in skeletal muscle, resulting in increased cyclic AMP and an activation of cyclic AMP-dependent protein kinase [28]. However, subsequent events leading to the regulation of protein turnover are not well studied in muscle. Phosphorylation of two intermediate filament proteins, desmin and vimentin, was observed in avian skeletal muscle cells in culture [29]. Post-receptor events have been examined in other tissues. The phosphorylation of at least 13 proteins in rat cardiac ventricular cells and the dephosphorylation of a single protein of molecular mass 21 kDa is stimulated by isoproterenol [17]. The response is rapid and the phosphorylations reach a maximum within 5 min. Three of the proteins have been identified and are troponin I, C-protein, and phospholamban, the modulator of the sarcoplasmic reticulum calcium-dependent pump ATPase [17].

Several processes altered by β-adrenergic stimulation are also implicated in the control of protein metabolism in muscle. Calcium transport across the plasma membrane (Ca^{2+} channel) and intracellular calcium movements are affected by β-agonists [30]. Calcium concentrations are known to be strongly linked to regulation of muscle protein degradation [31–33] as well as having a role in protein synthesis [34,35]. Furthermore, calcium is intimately involved with the regulation of the contractile elements of skeletal muscle [36–39]. The contractile activity of muscle is also influenced by β-agonists [12,16,40–42]. β-Agonists stimulate sodium/potassium pump activity [12,42]. The inter-relationship between pump activity and many metabolic functions emphasizes the complexity of the system and hence the difficulty in identifying the primary site of β-agonist action. Modulations similar to those described above may alter the function and activity of proteins and enzymes involved in protein turnover in skeletal muscle or may alter the cellular environment in a manner which results in stimulation of the mechanisms of protein accretion.

If the effect of β-agonists is direct, it is obvious that the post-receptor events in skeletal muscle must be different from those in smooth muscle and tissues such as liver. The link between receptor activation and influence on the rates of protein turnover and protein accretion is not present in the latter tissues [2,9]. Studies should capitalize on this key difference to determine post-receptor events in skeletal muscle.

Mechanism of action of muscle hypertrophy

Adult muscle maintains a constant size under normal conditions. Thus, the β-agonist must in some way influence the 'set point' of skeletal muscle, removing the normal controls responsible for maintaining a balanced state. The primary issue which must be addressed to ascertain the mechanism of action of β-agonists is whether the effect on protein turnover is direct or indirect. Research is hampered by the fact that the metabolic pathways and regulation of intracellular protein turnover in muscle and other cell types are largely unknown. Therefore, initial descriptive experiments are necessary to define the target area, as well as provide the rational bases for future mechanistic approaches.

Fig. 1 illustrates possible mechanisms of action which are discussed below. β-Agonists may bind directly to skeletal muscle membrane receptors and activate a sequence of events leading to protein accretion. Alternatively, muscle hypertrophy may be due to indirect mechanisms. The β-agonist may activate a non-muscle-cell β-receptor, leading to the production of hormone(s) or other factor(s). These factors may then act on the muscle or create an environment conducive to the stimulation of protein accretion.

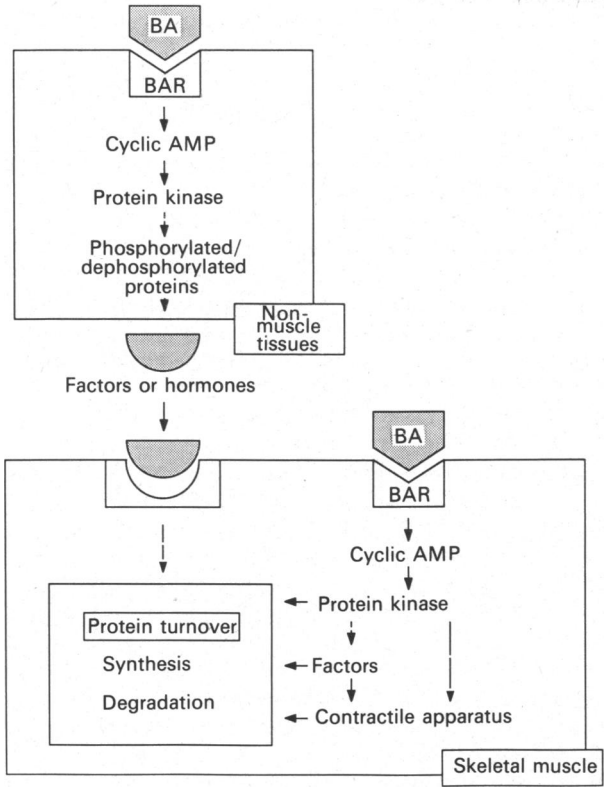

Fig. 1. Possible mechanisms of action for β-agonist-induced muscle hypertrophy

Key: BA, β-agonist; BAR, β-agonist receptor.

Indirect mechanisms

β-Receptors are present on almost every cell type. Many endocrine organs have a role in the regulation of muscle growth and β-agonists interact with several endocrine systems. Therefore, it is necessary to consider that β-agonists may promote muscle growth indirectly. There may be one or several primary target tissues for the initial β-agonist action.

Insulin. The interaction of insulin and β-agonists has been studied in some detail. Insulin is an anabolic hormone with potent effects on muscle protein metabolism. The release of insulin from the pancreas is stimulated by acute β-agonist treatment [43]. Insulin levels are reported to decrease or remain the same with chronic β-agonist treatment [2,6,8]. Muscle growth is stimulated by β-agonists in both severely diabetic rats [1,8] and in diabetic rats given a daily fixed dose of insulin [8] to circumvent the possible modulation of insulin levels. These data strongly suggest that alterations in circulating levels of insulin do not account for the β-agonist-induced muscle hypertrophy in normal animals.

Other hormones. The pituitary hormones play a crucial role in normal growth regulation. Growth hormone levels in sheep fed cimaterol for 6 weeks are reported to increase 2–3-fold but, surprisingly, plasma insulin-like growth factor 1 (IGF-1) levels decreased 34% [6]. No changes in growth hormone levels were observed in rats injected with clenbuterol twice daily for 16 days [2]. Thyroxine is also required for normal growth, although high concentrations are catabolic. Plasma T_4 increased 25% in lambs fed cimaterol for 6 or 12 weeks [3,6]. Plasma T_3 levels were not changed in these lambs [6] or in rats [2,16]. Glucocorticoids regulate β-agonist receptor number and sensitivity in muscle [44], and high concentrations promote potent muscle wasting [45,46]. However, plasma cortisol concentrations in lambs [6] or rats [47] were not altered. Muscles in β-agonist-fed hypophysectomized rats are larger than those of control hypophysectomized rats [48] suggesting that pituitary hormone secretion is not required for β-agonist-induced muscle hypertrophy. These hormone levels discussed above were measured after chronic treatment and thus the relationship to the anabolic action on muscle, which is an early response, is not clear.

β-Agonists are effective growth promoters in both male and female animals [9] as well as in castrated [5,6] and hypophysectomized [48] animals, indicating that gonadal steroid hormones are also not involved in β-agonist-stimulated muscle hypertrophy.

The effect of serum from β-agonist-treated rats on anabolic processes in muscle cells in culture was studied, to examine whether unidentified circulating hormones or factors were involved in β-agonist-induced muscle growth. This serum had the same anabolic activity (rates of protein synthesis, protein degradation, protein accretion) as normal rat serum [49].

Blood flow. One of the marked physiological responses to acute β-agonist treatment is peripheral vasodilation, which increases blood flow to skeletal muscles. This might provide extra substrates for muscle growth. Rothwell *et al.* [14] showed that chronic clenbuterol treatment of rats lowered blood flow in the hind leg muscle, although there was an initial increase. There is also an early increase in blood flow rate and oxygen uptake in the hind-quarters of cattle treated with clenbuterol [50]. However, this effect also declined within days. Since β-agonists only increase blood flow temporarily, it is unlikely that they exert an effect on muscle growth only through an alteration in blood flow.

Summary. These data do not identify an indirect mechanism for the anabolic action of β-agonists. The ability of β-agonists to stimulate muscle growth under all conditions of altered endocrine status argues against the involvement of these hormones and factors in the β-agonist mode of action. However, a subtle change may be present *in vivo* that is not appreciated in the current models used. For example, β-agonists may act in a paracrine manner, stimulate non-muscle cells present in whole muscle (connective tissue cells, smooth muscle cells etc.) to produce factors which regulate the growth of adjacent muscle cells. The lack of these components in model systems, such as cell culture, might explain the inability to demonstrate consistently activity of the β-agonist on muscle protein turnover *in vitro*.

Direct mechanisms

Direct coupling of the β-receptor to protein turnover has not been consistently demonstrated in muscle preparations *in vitro*, which possess biologically functional β-receptors and are capable of anabolic responses to a variety of agents. Research in this area is still preliminary and most of the information is only published in abstract form. Thus, these data are important but

Table 1. β-Agonist effects on protein turnover

This Table summarizes the findings from published reports of measured effects of β-agonists on protein turnover: (+), increase; (−), decrease; (0) no change.

	Synthesis			Degradation		
	(+)	(−)	(0)	(+)	(−)	(0)
In vitro						
Isolated muscle						
Avian			59,60		59,60	
Rat			59		51,52	59
Cultured muscle cells						
Avian	54,58				58	
Mouse			57			57
Rat			55,56,57		57	55,56
In vivo						
Avian			65		65	
Cattle					64	
Lamb			66		66	
Rat	2,61		9		9,61	
Pig	63			2		

should be interpreted with caution. Table 1 summarizes the published information to date concerning a direct effect of β-agonists on protein turnover.

Protein turnover. There were early reports in the literature suggesting that β-agonists modulated rates of protein turnover under certain conditions *in vitro* [51–54], but in general these early studies were never confirmed. Isoproterenol was reported to reduce the release of glutamine and alanine from incubated diaphram muscles, which suggested that this β-agonist inhibited protein degradation [51]. Isoproterenol also inhibited protein degradation in perfused hemicorpus in rats [52]. An interesting early report also observed that dibutyryl cyclic AMP stimulated myoglobin synthesis in avian muscle cultures (90–100 %) and increased the synthesis of total soluble protein (20–40 %) but only in cultures incubated in low calcium [54].

Since the discovery a few years ago that β-agonists are potent growth promoters, there has been renewed interest in this area. Protein synthesis, protein degradation, amino acid uptake and protein accretion have been measured in muscle cell cultures incubated in the presence of β-agonists. Zinterol [55] and cimaterol [56], over a wide range of concentrations, did not alter the anabolic activity in L8 or L6 muscle cells in culture respectively. In contrast, cimaterol at 1 μM, but not at higher concentrations, inhibited protein degradation in rat muscle cell cultures while cimaterol had no effect in MM14D mouse muscle cell cultures [57]. Protein synthesis was not affected by the β-agonist in either cell type [57]. Cimaterol, at high concentrations (1 μM) had minor effects on total cell protein accretion in cultured embryonic chicken muscle cells [58]. However, the β-agonist increased the myofibrillar protein myosin heavy chain protein 25–30 % and increased [^{14}C]leucine incorporation into myosin heavy chain.

Incubated isolated muscles from young rats have been used successfully for the study of skeletal muscle protein metabolism. However, it has been difficult to demonstrate the acute effect of β-agonists on protein synthesis or degradation in this model. Clenbuterol is reported to decrease protein degradation in the incubated skeletal muscle from chick, but had no effect on muscle from rats [59]. Clenbuterol did not affect protein synthesis in either muscle. More recently, cimaterol has been observed to inhibit protein degradation in incubated wing muscle from chicks [60]. Since similar effects were noted in the presence or absence of lysosomal inhibitors, it was proposed that β-agonists inhibited the non-lysosomal pathway of protein degradation. Cimaterol had no effect on protein synthesis in these muscles. It is clear that much needs to be done to refine these *in vitro* models. The apparent differences between rat and chick muscle are interesting and should be further explored.

Contractile elements. Contractile activity is one of the most potent physiological factors which influence muscle mass and it is well documented that β-agonists alter the contractile properties of muscle. Reduction of muscle contraction (e.g. denervation, weightlessness and disuse, etc.) results in a rapid wasting. Clenbuterol stimulates fibre hypertrophy in denervated rat soleus muscles [61]. The rats were treated with the β-agonist 3 days after denervation, at which time fibre atrophy was apparent, evidence of a true hypertrophy, not a blocking of the wasting effect. These results strongly suggest that β-agonists may mimic some parameter of neural control on muscle size. Zeman *et al.* [62] also investigated the effects of β-agonists on denervated muscle. Since treatment was initiated immediately following denervation, muscle growth was attributed to an inhibition of the wasting process.

Summary. If the effect of β-agonist-induced muscle hypertrophy is direct, it is unclear why the effect cannot be consistently demonstrated in the usual models *in vitro*. Some crucial component must be missing. Contractile activity in muscle, as mentioned above, is a necessary condition for the maintenance of a healthy muscle. β-Agonists affect both tension development and the speed of contraction of skeletal muscle [12,16,40–42]. Contractile activity and the attendant metabolic alterations may be the conditions which are lacking in the muscle experiments incubated *in vitro*, which prevent the expression of a direct effect of β-agonists on muscle protein turnover. Although there is some level of contractile activity in muscle cell cultures, it does not approximate the condition *in vivo*.

Effects on protein turnover *in vivo*

Whether protein anabolism is stimulated directly or indirectly by β-agonists, rates of protein turnover must be altered. Attempts have been made using models *in vivo* to establish which component of protein turnover is affected by β-agonist treatment.

Protein synthesis. Emery *et al.* [2] first reported that daily injections of clenbuterol and fenoterol (1 mg/kg) for 6 days increased muscle mass. The protein turnover rate *in vivo* was estimated using the short-pulse, large-dose, radioactivity labelled amino acid infusion method. The fractional rate of muscle protein synthesis increased without decreasing the fractional rate of protein degradation. In contrast, clenbuterol fed to young rats (200 μg/kg body wt.) for 4 or 8 days did not alter the fractional

rate of synthesis of muscle [9]. A similar method to determine the muscle protein turnover rate was used. Since there was a net increase in muscle protein mass, it was concluded that clenbuterol must have increased muscle growth by decreasing the rate of protein degradation. The conflicting results in the two above reports may be due to differences in the mode of drug administration, the dose of the drug, and/or the timing of the measurement. Interestingly, clenbuterol feeding was observed to increase skeletal muscle protein in denervated muscle by increasing the rate of protein synthesis [61].

If β-agonist-induced muscle hypertrophy occurs by stimulating protein synthesis in muscle, it is important to assess the effect of β-agonist treatment on the capacity and efficiency of protein synthesis as well as the site of action. Ractopamine has been shown to increase the rate of muscle α-actin synthesis *in vivo* by 50 % in grower pigs treated for 3 weeks [63]. Since the relative abundance of muscle α-actin mRNA increased 2–3-fold, this suggests that β-agonists enhance protein synthesis at a pre-translational level.

Protein degradation. It is also difficult to accurately quantify the rate of protein degradation *in vivo*. The tracer method in the above studies can be used to calculate the rate of protein degradation as the difference between estimated synthetic rate and net muscle protein accumulation over a period of time. This estimation, by the inherent nature of the method, is subject to error, especially when applied to large animals. Measurement of 3-methylhistidine excretion has also been used as an indication of protein degradation. This method suggested that protein degradation decreased in young veal calves in response to β-agonist treatment [64]. Continuous infusion of [^{14}C]tyrosine is another method used to estimate the rate of muscle protein turnover, and showed that cimaterol treatment of growing chickens for 1 week decreased the fractional degradative rate in the breast and leg muscle but did not alter the fractional rate of synthesis [65]. The fractional degradative rate also decreased in several muscles of clenbuterol-treated lambs with no effect on the rate of protein synthesis [66].

The steps in the proteolytic pathway are not as clearly defined as they are for protein synthesis. Proteases are necessarily involved, and an indirect way to investigate changes in intracellular degradation is to assess qualitatively the activity of lysosomal and non-lysosomal proteases in the tissue. β-Agonists appear to down-regulate lysosomal protease activity in the muscle of β-agonist-treated animals. Cathepsin B activity is reduced in muscle, but not liver [67], from lambs fed cimaterol and in muscle from lambs fed L-644,969 [68]. Cathepsin B activity also decreased in muscle from cimaterol-treated chickens [69]. Clenbuterol treatment for 1 week did not alter cathepsin B or D activity in the EDL or gastrocnemius muscles but elevated their activity in the soleus muscle of rat [8]. After 2 weeks of treatment, cathepsin B activity decreased in the gastrocnemius and the EDL muscles, and increased in the rat soleus muscle [7]. These alterations with time probably reflect the adaptations of the degradative machinery of the muscle to chronic β-agonist treatment.

The non-lysosomal pathway is also an important route for normal muscle protein turnover. The μM-calcium-dependent proteinase is considered to have a special role in skeletal muscle protein degradation. The activity of this protease decreased 55–70 % in the longissimus dorsi muscle, which increased 30 % in size with cimaterol treatment [70]. Also, a 68 % increase in the activity of the calcium-activated proteinase inhibitor (calpastatin) was observed in the longissimus dorsi muscle of lambs treated with L-644,969 for 6 weeks [68]. These above data suggest that β-agonists may decrease muscle protein degradation in several species of animals.

Summary. The effect of β-agonists on protein turnover, whether direct or indirect, is most likely a complex modulation which changes with time and physiological conditions. To fully clarify the actions of β-agonists, it is crucial to obtain a stringent timecourse analysis in which both components of protein turnover are measured.

LIPID METABOLISM

Reduction of body fat and increased energy expenditure are among the most pronounced physiological effects of chronic β-agonist treatment. Decreased body fat may be a consequence of increased fat mobilization from adipose tissue, decreased fat synthesis in adipose tissue and liver, or a combination of both. The ability of adrenaline to modulate lipid metabolism directly in liver and adipose tissue has been demonstrated in many animal species. Binding of β-agonists to adipose tissue adrenergic receptors activates in turn adenylate cyclase, cyclic AMP levels, the protein kinase cascade, and leads to the activation of the hormone sensitive lipase and triacylglycerol hydrolysis. In liver and adipose tissue, elevation of intracellular cyclic AMP concentrations by β-adrenergic stimulation may inhibit fatty acid synthesis by attenuating the key regulatory enzymes [71]. There are significant differences in the regulation of lipid metabolism from species to species. Thus, the action of β-agonists in reducing fat deposition may also vary. Therefore, we will discuss each species separately.

β-Receptor subtype

The β-receptors in adipocytes were originally believed to be β_1 [18,72] while those in liver have been classified as β_2 [73]. Recent discovery of highly adipose-tissue-selective β-agonists provided good evidence that the functional receptors in the adipocytes are distinct from either β_1 or β_2 [74,75]. Since adipose tissue and liver play important but different roles in the lipid metabolism among different animal species, the actions of β-agonists on lipid metabolism could conceivably vary according to the receptor selectivity of the compound used, as well as the species of the target animals studied.

Mechanism of action

The lipolytic and anti-lipogenic activities of β-agonists can be readily assessed directly *in vitro* in incubated adipocytes and hepatocytes. Acute lipolytic responses *in vivo* are commonly determined by monitoring the blood levels of non-esterified fatty acids or glycerol following drug administration. Acute lipogenic responses *in vivo* are more difficult to quantify directly, especially in large animals. Chronic adaptive changes in the activity of lipogenic enzymes have been used as an index for altered lipogenic activity *in vivo*. Due to the recent availability of long-acting, orally active synthetic β-agonists, the chronic effects of β-agonists on lipid metabolism can now be more readily studied.

Rodents. The effects of β-agonists on lipid metabolism have been extensively investigated in the laboratory rodent species. β-Agonist treatment significantly reduces body fat in rats [2,76,77]. It is well documented that β-agonists can stimulate lipolysis in rat adipocytes and inhibit fatty acid synthesis in hepatocytes and adipocytes *in vitro*. Both liver and adipose tissue are the site for fatty acid synthesis *de novo* in rats [78]. β-Agonists are also observed to reduce the proliferation of preadipocyte in culture [79]. This suggests that an alteration in cell number may also have a role in β-agonist-induced fat reduction.

Cimaterol fed to growing rats for 4 weeks did not alter the activity *in vivo* of fatty acid synthesis in liver and white adipose tissue as determined by ^3H_2O incorporation [76]. The β-agonist L-640,033 fed for 1 week reduced epididymal fat pad weight and significantly decreased the total activity of lipogenic enzymes in the liver, but not in the epididymal fat pads [77]. These data suggest that chronic administration of β-agonists enhances fat mobilization from adipose tissue without altering lipogenesis in this tissue. The absence of anti-lipogenic activity in adipose tissue *in vivo* is surprising, but may be explained, in part, by the observation that low concentrations of β-agonists enhance fatty acid synthesis from glucose in isolated rat adipocytes, if insulin is present at physiological concentration (Y. T. Yang and L. S. Firman, unpublished work). High concentrations of β-agonists inhibit lipogenesis with or without the presence of insulin. Earlier, Saggerson [80] obtained similar results with adrenaline and proposed that adrenaline may increase the energy expenditure of rat adipocytes, thereby relieving the feed-back inhibition of fatty acid synthesis by excess cytosolic NADH accumulation.

Meat-producing mammals. β-Agonist feeding markedly reduces the adipose tissue mass in several meat-producing animals, including sheep, pigs and cattle [3–5]. Long term feeding of cimaterol to growing lambs markedly decreased carcass fat and elevated plasma non-esterified fatty acid concentrations, suggesting enhanced lipid mobilization [6]. However, the fatty acid synthetic activity of the subcutaneous adipose tissue increased in β-agonist-fed sheep [81,82].

Carcass fat decreased at all sites in cattle fed clenbuterol for 50 days [83,84]. The activity of lipogenic enzymes and fatty acid synthesis in the adipose tissue after chronic treatment was measured *in vitro*. These activities were reduced in the subcutaneous adipose tissue, but not in the intramuscular or perirenal adipose tissues.

Clenbuterol reduced carcass fat content in pigs [85]; however, there has been difficulty in demonstrating a direct lipolytic activity of this compound in pig adipose tissue *in vitro* [86,87]. Thus, Mersmann [87] suggested that β-agonists may indirectly reduce fat deposition in pigs. In contrast, ractopamine, which also decreases carcass fat in pigs [88], stimulates lipolysis and inhibits lipogenesis in isolated adipocytes from pig [86]. The activity of enzymes involved in lipogenesis and fatty acid synthesis were reduced in adipose tissue from ractopamine-fed pigs and lipolytic activity was increased [89]. The β-agonists L644,969 and cimaterol are also potent lipolytic agents in pig adipose tissue both *in vitro* and *in vivo* [4,5,90]. Thus, it appears that in general β-agonists reduce fat in pigs by directly increasing lipid mobilization. The encountered difficulties may suggest that the selectivity of the β-receptor in pig adipose tissue may be different in some respects from other species.

The effects of β-agonist treatment on hepatic lipid metabolism in pigs, sheep or cattle have not been reported. However, the capacity for hepatic fatty acid synthesis is very limited in these animals [91–93].

Chickens. The reduction of body fat by β-agonists in broiler chickens was relatively small [94] and not consistently observed. In contrast to mammals, lipolysis in chicken adipocytes is not responsive to adrenaline stimulation [95]. However, lipogenesis in the liver, the major organ for fatty acid synthesis *de novo* in chickens, is very sensitive to adrenaline inhibition [96,97]. Thus, β-agonist treatment may reduce the body fat of chickens by inhibiting fatty acid synthesis in the liver without a direct effect on adipose tissue.

Summary. These data indicate that direct stimulation of lipid mobilization from adipose tissue may be the major common mechanism for β-agonist-induced reduction of body fat in mammalian species, but that liver may be the more important organ in chickens.

THERMOGENESIS

β-Agonists may reduce body fat by stimulation of lipid mobilization and by inhibition of lipid synthesis. In addition, these compounds enhance overall energy expenditure of the animal via non-shivering thermogenesis to dissipate the excess energy which is not stored in the adipose tissue.

Brown fat

Brown adipose tissue plays an important role for cold- and diet-induced thermogenesis in rodents [98,99]. Brown adipose tissue is different both morphologically and functionally from white adipose tissue. It is more vascular and has a very rich sympathetic innervation. The brown adipocytes are characterized by the presence of a 32 kDa protein, also known as thermogenin, in the inner membrane of mitochondria [100]. This protein forms the unique proton conductance pathway which allows proton re-entry into the mitochondrial matrix without coupling to ATP synthesis. Thus, respiration and heat production can proceed without restraint and is not limited by cellular ATP requirements. Thermogenin has an inhibitory purine nucleotide binding site [101]. Specific binding of GDP to this mitochondrial protein has been used as an index of the thermogenic state of brown adipose tissue. Both β- and α-adrenergic stimulation can elicit thermogenic responses in brown adipose tissue, although α-receptors may play a minor role [102]. Acute β-agonist administration causes a rapid increase in brown adipose tissue temperature, increase in oxygen consumption, and an increase or unmasking of mitochondrial GDP binding sites [103]. Chronic β-agonist treatment increased brown adipose tissue protein mass and cell proliferation, the concentration of GDP-binding protein and the total thermogenic capacity [104]. Thus, chronic β-agonist treatment enhances the thermogenic capacity of brown fat in rodents.

Brown fat β-receptor

The β-receptors in brown adipose tissue were initially

classified as β_1 based on the potency ranking of selected adrenergic agonists to stimulate brown fat cell respiration [105]. Later, it was found that injection of either β_1- or β_2-selective agonists resulted in stimulation of brown fat thermogenesis in rats. The effect was inhibited by both β_1- or β_2-selective antagonists respectively, indicating the involvement of both receptor subtypes in the induction of thermogenesis [103]. Analysis of ligand binding characteristics of brown adipocyte membranes also suggested the presence of both β_1 and β_2 receptors in brown adipose tissue [106]. However, the recent discovery of adipose-selective β-agonists, as discussed above, has provided reasonable evidence that β-receptors in brown and white adipose tissues are distinct from the classical β_1 and β_2 types.

Other thermogenic organs

Adrenergic-induced thermogenesis has been observed in several animal species [107,108]. Brown adipose tissue is most likely the major site of this thermogenesis in some rodent species. The existence or the quantitative importance of brown fat in other animals is more dubious.

Administration of β-agonists to adult human subjects resulted in a significant increase in energy expenditure. However, the estimated potential contribution from adipose thermogenesis could account for only a very small fraction of total increment [109]. Skeletal muscle, due to its bulk, may be an important site of adrenergic-induced thermogenesis in human as well as in other animals. Adrenergic agonists have been demonstrated to increase oxygen consumption in skeletal muscle [110–113]. Although β-receptors in skeletal muscle are shown to be predominantly β_2 [22–25], Challiss *et al.* [114] recently compared the activity of various β-agonists and antagonists on the glycogen synthesis and lactate production in incubated muscle, and concluded that the 'β_2' receptor in skeletal muscle is different from the classical β_2 receptor in smooth muscle. Thurlby & Ellis [110] determined the effects of BRL26830, an adipose-selective adrenergic agonist, and β-antagonists on oxygen uptake in the perfused hindlimbs of rats. They suggested that the β-receptors responsible for thermogenesis in the skeletal muscle may be similar to the receptors in brown fat cells. Interestingly, feeding BRL26830 to normal mice significantly reduced adipose tissue mass but had no effect on skeletal muscle mass [115]. Together, these data imply that there may be different β-receptors in the skeletal muscle, and their functional responses may be compartmentalized.

Summary

Although skeletal muscle has been recognized as a potential thermogenic organ responsive to adrenergic stimulation, the mechanisms of specific thermogenic regulation in this tissue are still unknown. To determine the potential use of thermogenic β-agonists, several issues have to be addressed, including the identification of thermogenic target organs, the thermogenic potential and the receptor specificity of these organs.

HYPERGLYCAEMIA

Adrenaline has long been known as a hyperglycaemic hormone. Sympathetic stimulation or administration of adrenaline causes a rapid elevation of blood glucose [113,116]. This hyperglycaemic response is the result of increasing hepatic glucose output and suppression of insulin secretion, primarily by the action of the α-adrenergic component of adrenaline. The discovery of long-acting β-agonists has afforded the opportunity to examine the effects of chronic β-agonist treatment on glucose metabolism and the findings are surprising.

Acute effects

Acute effects of β-agonists on blood glucose levels are related to the interactions of β-agonists with several parameters of insulin regulation. β-Agonists such as isoproterenol, when given acutely, increase plasma insulin concentration with minimal changes in blood glucose [117]. The β-adrenergic receptors on the pancreatic islet of Langerhans β-cells were shown to be of β_2 subtype [117]. Following acute β-agonist treatment, cyclic AMP-dependent protein kinase phosphorylates the insulin receptor and lowers the receptor tyrosine kinase activity [118,119]. Acute treatment with isoproterenol has also been shown to produce insulin resistance in adipocytes *in vitro* [120].

Chronic effects

It is interesting to find that, when given chronically, β-agonists are extremely potent anti-hyperglycaemic agents. Chronic administration of BRL26830 to genetically diabetic mice decreased blood glucose to normal levels, and increased plasma and pancreatic insulin concentrations [121]. This anti-hyperglycaemic response was explained in part by the increase in insulin secretion. β-Agonists also effectively improve hyperglycaemia in genetic or diet-induced obese mice and rats [114,122,123], and in glucocorticoid-induced hyperglycaemic rats (Y. T. Yang & J. B. Spencer, unpublished work). In the latter case, there was no increase in blood insulin levels, suggesting an improvement in insulin sensitivity. More subtle but consistent changes in the insulin function were also observed in the normal animals treated with β-agonists. Lambs fed cimaterol for several weeks had lower circulating insulin and glucose concentrations [6]. The fasting glucose tolerance in insulin-resistant obese Zucker rats is improved by chronic treatment with BRL26830 [123]. There was improvement in whole body insulin sensitivity, increase in glucose utilization rate, and a small increase in endogenous glucose production. Insulin sensitivity in soleus muscle was also enhanced in insulin-treatment obese Zucker rats treated with BRL26830 [114].

It is clear that chronic β-agonist treatment can dramatically improve overall insulin sensitivity, especially in animals with inherent insulin resistance. However, the tissues and the β-receptor types involved and the mechanism of this action are not yet understood. Tissue sensitivity to insulin can be improved by increasing insulin receptor affinity, number, receptor–effector coupling efficiency or the post-receptor metabolic capacity. Gold thioglucose-induced obese mice are insulin-resistant and have reduced binding of insulin receptors and receptor kinase activity in brown adipose tissue and skeletal muscle [122]. Chronic treatment of these animals with BRL26830 increased receptor binding and receptor kinase activity, but the effect was not observed in tissues from lean control animals.

Obesity is often associated with insulin resistance and hyperglycaemia. Weight reduction improves insulin sensitivity and the diabetic state. However, the anti-diabetic

activity of β-agonists appears to be independent of the anti-obesity response. The anti-diabetic response is evident within one day of treatment (Y. T. Yang & J. B. Spencer, unpublished work). In addition, hyperglycaemic obese mice treated with low doses of β-agonist had markedly reduced blood glucose concentrations without a reduction in adipose tissue mass.

Summary

The anti-hyperglycaemic effect of β-agonists is striking. Although the mechanism of action is still not known, the wealth of information available in the diabetic area should greatly enhance the progress in resolving this important problem.

IMPLICATIONS

Muscle wasting is a critical health issue. Immobilization of muscles, as in casting of appendages, disuse due to paralysis or lengthy bed rest, muscular dystrophy, and burn- or cancer-induced cachexia, all result in muscle atrophy. Weightlessness also promotes muscle protein loss. The remarkable anabolic activity of these β-agonists in skeletal muscle suggests the great therapeutic potential of these compounds. It remains to be determined whether β-agonists can prevent muscle wasting in these situations.

Adipose tissue metabolism and thermogenesis studies indicate the importance of selective β-agonists for treating human obesity. Several compounds of this class are currently being developed as anti-obesity agents for humans. There is also potential utility for β-agonists in animal production. These compounds produce leaner meat, which is of interest to the consumer and the increase in feed efficiency is of prime economic importance to the producer. β-Agonists with selectivity for thermogenesis would not be desirable in this case because feed efficiency would be reduced.

The drastic improvement in non-insulin-dependent diabetes in laboratory animal models is intriguing. Since a large percentage of the human population suffers from this Type II diabetes, a therapeutic agent such as this would certainly be invaluable.

The practical application of these compounds will be determined largely by their efficacy and side-effects. The biological actions of these drugs are complex. One encouraging development is the possibility of a greater than anticipated β-receptor diversity among different tissues, which would allow for the design of efficacious therapeutic agents with minimum unwanted side-effects.

CONCLUSIONS

To date there is no compelling evidence to implicate any anabolic hormone as a mediator of the β-agonist-induced skeletal muscle growth. The preliminary data from studies *in vivo* measuring the effect of β-agonists on protein turnover are promising. However, the development of a consistent model *in vitro* is crucial for progress in this area. The identification of a unique β-receptor in adipose tissue provides a new avenue for research into the biochemical control of lipid and energy metabolism. Also, the therapeutic applications of β-agonists as anti-hyperglycaemic agents will be invaluable for understanding the aetiology of the diabetic processes.

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