Quantitative analysis of beta-adrenergic receptor subtypes in pig tissues.

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Quantitative analysis of β-adrenergic receptor subtypes in pig tissues[1]

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ABSTRACT: The density and distribution of β_1- and β_2-adrenergic receptors (βAR) in porcine adipocytes, skeletal muscle, heart, lung, and liver were investigated using competitive displacement of ligand binding with subtype-selective ligands. Three experimental approaches were used to estimate the distribution of βAR subtypes in adipocytes. Two approaches involved simultaneous linear regression analysis of multiple competitive displacement curves with the β_1AR-selective antagonist CGP 20712A and the β_2AR-selective ligand BRL 37344. For the third approach, radioligand saturation assays were perfomed using a concentration of CGP 20712A that completely blocked the β_1AR. All three approaches indicated the presence of multiple βAR subtypes in porcine adipocytes and gave similar estimates for the proportion of these subtypes. Saturation assays in the presence of the β_1AR blocker CGP 20712A were conducted to determine the distribution of the βAR subtypes in skeletal muscle, heart, lung, and liver. The proportions of the β_1AR and β_2AR were 81:19, 59:41, 72:28, 58:42, and 50:50 for adipose, skeletal muscle, heart, lung, and liver, respectively. These estimates based on receptor protein were consistent with published estimates of mRNA abundance in pig tissues but differ from estimates for other species. The predominance of β_1AR in adipocytes and skeletal muscle may contribute to the reduced efficacy of select βAR agonists in pigs compared to other species because most of the ligands evaluated in growth studies are purported to be β_2AR selective. The density of the βAR varied among tissues in the following order: heart = lung > adipocytes > skeletal muscle ≥ liver.

Key Words: Adipocytes, β-Adrenergic Receptor, Liver, Muscle, Pigs

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Introduction

Three subtypes of β-adrenergic receptor (**βAR**) have been cloned from multiple species, and most tissues express more than one subtype (Strosberg, 1997). All three subtypes signal through the G-protein Gs to activate adenylyl cyclase, implying some redundancy in the regulation of cell responses. Although redundancy may exist, unique roles for each subtype is likely as well. Each subtype exhibits pharmacokinetic differences (Tate et al., 1991), and evidence for signal compartmentalization (Hollenga et al., 1991) and activation of alternative signaling pathways (Luttrell et al., 1998) suggests a complexity that is just beginning to be appreciated.

Several βAR ligands are approved for commercial use in livestock in select countries (Moody et al., 2000). The next generation of ligands may target specific βAR subtypes and cellular events. Determination of the distribution and role of βAR subtypes in the pig has been hampered by the uncertainty of subtype-selective ligands due to pharmacology that is particular to a species (Coutinho et al., 1992; Liang and Mills, 2001; Mersmann et al., 1993). The pattern of βAR mRNA in pig tissues indicates a distribution that differs from that of the rat, with a predominance of β_1AR in the major tissues (McNeel and Mersmann, 1999). In order to confirm that βAR protein follows the pattern of mRNA abundance, we have identified β_1- and β_2AR-selective ligands from cloned receptors expressed in Chinese hamster ovary cells (Cao, 1998; Liang and Mills, 2001). We used these ligands to quantify the distribution of β_1AR and β_2AR subtypes in porcine adipocytes, skeletal muscle, heart, lung, and liver.

Materials and Methods

Tissue Collection and Processing. Crossbred pigs (PIC line 337 sires × York-Landrace dams) from the Purdue University research farm were fed a corn- and soybean-based ration to meet nutritional requirements and were killed by electrical stunning and exsanguination at the Purdue University Meat Labora-

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tory. Subcutaneous adipose tissue (mixture of middle and outer layers) and longissimus muscle were taken from the 10th to 12th rib of market weight barrows. Heart, lung, and liver were obtained from barrows weighing 20 to 30 kg. Muscle, heart, lung, and liver were quickly cut into pieces of approximately 0.5 cm^3, frozen in liquid nitrogen, and stored at –80 C. Adipose tissue was transported to the laboratory in 37 C buffered saline (0.15 M NaCl + 1 m*M* HEPES, pH 7.4) for isolation of adipocytes within 15 min. Adipocytes were prepared by collagenase digestion as described (Liu et al., 1989). Briefly, adipose tissue was sliced to less than 0.5 mm with a Stadie-Riggs microtome (Thomas Scientific, Swedesboro, NJ), minced and washed through a polypropylene screen with the buffered saline. Minced tissue (5 to 6 g) was incubated in 15 mL of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 1.25 m*M* $CaCl_2$, 0.5 m*M* ascorbic acid, 10 m*M* HEPES, 5 m*M* glucose, 3% BSA. and 0.67 mg/mL collagenase (Type I, Worthington Biochemical, Freehold, NJ) for 25 to 30 min at 37 C with orbital shaking (95 rpm). Digested tissue was filtered sequentially through 1,090- and 580-μm polypropylene screens, followed by three washes with Krebs-Ringer buffer without collagenase. Cells were suspended in 10 to 20 mL of a hypotonic lysing buffer (5 m*M* Tris·HCl and 5 m*M* EDTA with 2 μM leupeptin and pepstatin) for membrane preparation.

Membrane Preparation. The lysed adipocytes were homogenized using a Wheaton glass grinder (Fisher Scientific, Pittsburgh, PA) with eight to nine strokes and centrifuged at 1,000 × *g* for 5 min at 4 C. The fat layer was discarded and the supernate was transferred to a clean tube and centrifuged at 100,000 × *g* for 60 min at 4 C. The pellet was washed in incubation buffer (50 m*M* Tris·HCl, 5 m*M* $MgCl_2$, 1 m*M* EDTA, pH 7.4, 2 μM leupeptin, and 2 μM pepstatin) and centrifuged again at 48,000 × *g* at 4 C for 20 min. The final pellet was suspended in the incubation buffer and stored at –80 C. Skeletal muscle membranes were prepared with a modification of the procedure described (Spurlock et al., 1993). All centrifugations were performed at 4 C. Briefly, approximately 5 *g* of cubed tissue were homogenized on ice with a Tissuemizer (Model SDY-1610, Tekmar, Cincinnati, OH) in 10 to 15 mL of the hypotonic lysing buffer. Homogenates were filtered through a 1.1-mm polypropylene screen and centrifuged at 1,000 × *g* for 20 min. The supernatants were sequentially centrifuged at 10,000 × *g* for 20 min and 100,000 × *g* for 60 min. The 100,000 × *g* pellet was washed and suspended in incubation buffer as for adipocytes and stored at –80 C. Heart, lung, and liver membranes were prepared as described for skeletal muscle except the 10,000 × *g* centrifugation was omitted (Sano et al., 1993). Membrane protein was determined by Lowry (Lowry et al., 1951). Membrane fractions consist of plasma membranes and subcellular components and are termed *microsomal* membranes. These preparations are enriched in the plasma membrane markers 5′nucleoltidase and adenylyl cyclase activity (unpublished data).

Radioligand Binding. Competition and saturation binding to the βAR were conducted using [^{125}I]iodocyanopindolol (**[^{125}I]CYP**) (2,000 Ci/mmol, Amersham, Piscataway) as radioligand. Tissue membranes (10 to 50 μg in incubation buffer) were incubated in duplicate in 17- × 100-mm polypropylene tubes in a final volume of 0.15 mL at 37 C for 60 min. Nonspecific binding was determined in the presence of 0.1 m*M* (–)isoproterenol and was subtracted from total binding to give specific binding. Incubations were processed as described (Liang et al., 2000).

Data Analysis. Binding parameters and the fitted models were determined using the weighted least-squares nonlinear regression analysis computer program Kell (BIOSOFT, Cambridge, UK). The subprogram Radlig was used to analyze each individual binding curve and Ligand was used to simultaneously analyze multiple competition curves. Binding curves were tested routinely for the best fit to one-, two-, or three-site models. The parameters from the best-fit model were reported. Tissue differences for binding parameters were analyzed with least squares analysis of variance using a completely random design (SAS Inst. Inc., Cary, NC, 1985). Individual tissue differences were separated with the Student-Newman-Keuls test.

Results

The β_1AR and β_2AR are the predominant subtypes expressed in pig tissues, whereas β_3AR is expressed poorly in most tissues, except adipose, in which it represents 7% of the βAR mRNA abundance (McNeel and Mersmann, 1999). Therefore, quantifying the contribution of the β_1AR and β_2AR will likely account for most of the βAR in pig tissues. We have identified CGP 20712A (**CGP**) and BRL 37344 (**BRL**) as having at least 100-fold selectivity for the β_1AR and β_2AR, respectively, using cloned porcine receptors (Cao, 1998; Liang and Mills, 2001). Furthermore, the affinity of the cloned β_1AR and β_2AR expressed in cultured cells for [^{125}I]CYP was similar, which should make this radioligand ideal for determining subtype distribution. It was important, however, to confirm that [^{125}I]CYP was not selective for the βAR subtypes expressed in pig adipose tissues. To determine selectivity, CGP competition experiments were conducted at different concentrations of [^{125}I]CYP, as described (McGonigle et al., 1986). Displacement curves modeled best to two sites, reflecting the presence of multiple βAR subtypes (Figure 1). The proportion of βAR subtypes for each curve was obtained and the percentage of the high-affinity sites (Rh; β_1AR) increased from 31 to 61% as the concentration of [^{125}I]CYP increased. The progressive shift in the estimated proportion of βAR subtypes is interpreted to reflect significant subtype selectivity by the radioligand (McGonigle et al,. 1986). Experiments were repeated with BRL as competitor and

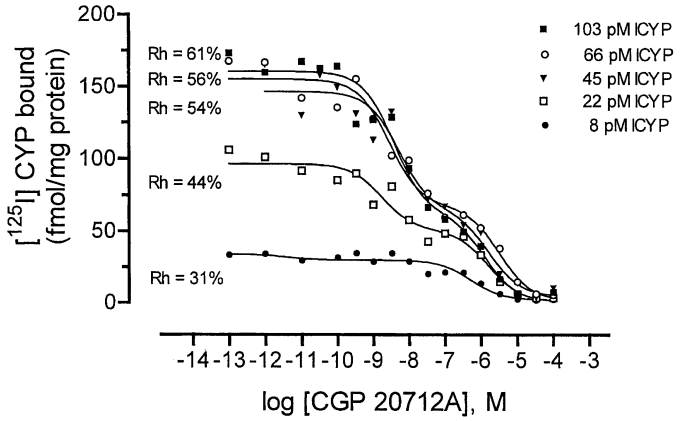

Figure 1. Competitive displacement of specific [^{125}I]CYP binding by the β_1-adrenergic receptor-selective antagonist CGP 20712A. Five concentrations of [^{125}I]CYP were incubated with porcine adipocyte membranes in the presence of increasing concentrations of CGP 20712A. The competition curves were analyzed using K_d values of 14.5 p*M* (β_2AR) and 19 p*M* (β_1AR) estimated from pβAR in CHO cells. The proportion of high-affinity receptors (Rh; β_1AR) calculated from each curve are shown.

three concentrations of [^{125}I]CYP (Figure 2). The same pattern was not obvious (Rl; β_1AR), but a more limited range of [^{125}I]CYP was used. For both CGP and BRL, the proportion of β_1AR approached similar values as the concentration of [^{125}I]CYP increased toward saturation (60 to 70% β_1AR). The apparent selectivity exhibited by adipose tissue βAR was not expected based

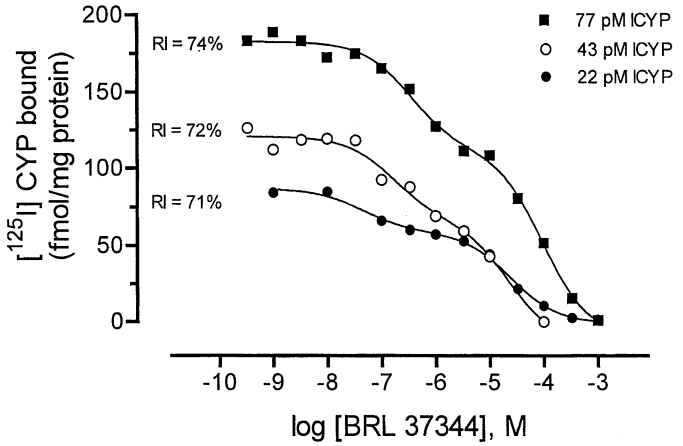

Figure 2. Competitive displacement of specific [^{125}I]CYP binding by BRL 37344. Three concentrations of [^{125}I]CYP were incubated with porcine adipocyte membranes in the presence of increasing concentrations of BRL 37344 and 100 μ*M* Gpp(NH)p. The competition curves were analyzed using K_d values of 14.5 p*M* (β_2AR) and 19 p*M* (β_1AR) estimated from pβAR in CHO cells. The proportion of low-affinity receptors (Rl; β_1AR) calculated from each curve are shown.

on the kinetics determined with the cloned βAR in culture. These data indicated that accurate estimates of the proportion of the βAR subtypes could only be obtained by simultaneous analysis of the multiple competition curves for CGP and BRL (Kell; BIOSOFT). These data are presented under the heading Competition Assay in Table 1. Several points can be made from these data. First, the results are consistent for both CGP and BRL. The binding of [^{125}I]CYP to the high- and low-affinity sites (presumably the β_1AR and β_2AR) in porcine adipose tissue is selective. The [^{125}I]CYP exhibited a 6- to 18-fold higher selectivity for the porcine β_2AR than for the porcine β_1AR (Table 1). Second, the estimated proportion of β_1AR and β_2AR in the adipocytes were 73:27 and 83:17 for CGP and BRL, respectively as competitors (Table 1). Finally, the affinity estimates (Ki) of the adipose βAR for CGP and BRL were determined (Table 1). The Ki for CGP was 160-fold lower for the porcine β_1AR than for the β_2AR (2.6 vs 418 n*M*), whereas the Ki for BRL was 3,000-fold lower for the porcine β_2AR than for the β_1AR (5.3 vs 16,050 n*M*). The selectivity for each ligand is consistent with expectations based on data from cloned pig βAR, except for the affinity estimate of the β_2AR for BRL, which was 122 n*M* for the cloned receptor (Liang and Mills, 2001). In order to reconcile the difference, BRL displacement of [^{125}]CYP binding to adipocyte membranes was conducted in the presence of 100 n*M* CGP to block the porcine β_1AR. The CGP concentration was calculated to block the β_1AR but not the β_2AR at ~60 pM [I^{125}]CYP using the Cheng and Prusoff formula; $K_i = IC_{50}/(1 + [\text{radioligand}]/K_d)$ (Cheng and Prusoff, 1973). As shown in Figure 3, competition curves fit a one-site model and yielded a K_i for BRL of 139 n*M* (Table 1). This latter value is similar to the estimate obtained in CHO (Chinese hamster ovary) cells, suggesting that the estimate from the simultaneous analysis was less accurate.

In order to confirm the proportion of the β_1AR and β_2AR determined by simultaneous nonlinear regression analyses, we conducted saturation experiments in the presence and absence of 100 n*M* CGP. Saturation binding of [^{125}I]CYP to adipocyte membranes in the absence of CGP modeled to one site (Figure 4). Scatchard plots were linear with a calculated K_d of 17 ± 6 p*M* and Bmax of 232 ± 45 fmol/mg membrane protein. In the presence of CGP the Bmax for the β_2AR was approximately 40 ± 6 fmol/mg membrane protein. Scatchard plots, however, were inconsistent, likely due to the low density of βAR when the β_1AR was blocked. Maximal binding, therefore, was estimated from the saturation curve rather than the Scatchard plot (Figure 4). The proportion of the β_1AR and β_2AR was estimated to be 81:19 in porcine adipocytes (Table 1). These estimates are in good agreement with the estimates from the competition experiments done with CGP and BRL.

We showed that accurate estimates of the proportion of βAR in porcine adipocytes could be obtained with

Table 1. Distribution of β-adrenergic receptor subtypes in adipocytes determined by different approaches

Item	Competition assay		Saturation assay[b] (n = 4)	BRL 37344 +CGP20712A[c] (n = 2)
	CGP 20712A[a]	BRL 37344[a]		
Bmax (β_1), %[d]	73 ± 18	83 ± 15	81 ± 2	
Bmax (β_2), %[d]	27 ± 12	17 ± 14	19 ± 2	
K_i (β_1), nM[e]	2.6 ± 0.7	16,050 ± 2,560		
K_i (β_2), nM[e]	418 ± 86	5.3 ± 2.4		139 ± 48
K_d (β_1), pM[f]	64 ± 17	57 ± 13		
K_d (β_2), pM[f]	11.2 ± 2.6	3.2 ± 1.2		

[a]Values were determined by simultaneous nonlinear regression analysis of multiple [^{125}I]CYP competition curves using the β_1AR selective antagonist CGP 20712A and the β_2AR selective ligand BRL 37344 (Kell program, BIOSOFT).
[b]Saturation assays were conducted in the presence or absence of 100 nM CGP 20712A. The receptor proportions were calculated from the Bmax values of the paired saturation curves.
[c]The K_i of BRL 37344 for the porcine β_2AR was determined from competition curves with BRL 37344 in the presence of 100 nM CGP 20712A.
[d]Percentage of total βAR.
[e]Dissociation constant of ligand.
[f]Dissociation constant of [^{125}I]CYP.

subtype-selective ligands using competition or saturation assays. Therefore, we elected to conduct saturation assays to estimate the proportion of βAR subtype in porcine skeletal muscle, heart, lung and liver. Saturation experiments were conducted as for adipocytes in the absence and presence of 100 nM CGP with membranes from each tissue and data are summarized in Table 2. For all tissues, specific binding was saturable in the absence of CGP, all curves fit a one-site model, and Scatchard plots were linear. In the presence of CGP, specific binding of [^{125}I]CYP to tissue membranes was saturable, but Scatchard plots could not be reliably analyzed. Therefore, maximal binding was estimated from the saturation curves as was done for adipocytes. Skeletal muscle and liver were particularly problematic. Nonspecific binding even in the absence of CGP was 60 to 80% of total binding. High background was a composite of low specific binding and high nonspecific binding, each measured as CPM bound per milligram of protein. The high background could not be reduced by increased washing, prior soaking of the membranes with 10% polyethylenimine, or by sonicating the membranes. By contrast, nonspecific binding in heart and lung was less than 15% of total binding. Based on the Bmax estimates from the paired saturation curves (± CGP), the proportions of β_1AR and β_2AR were calculated to be 59:41 (skeletal muscle), 72:28 (heart), 58:42 (lung), and 50:50 (liver). Densities of total βAR were 47, 493, 450, and 24 fmol/mg membrane protein in the porcine skeletal muscle, heart, lung and liver, respectively. Estimates of the affinity of the mixed population of βAR for [^{125}I]CYP were 75, 69, 38, and 46 pM for the porcine skeletal muscle, heart, lung and liver, respectively.

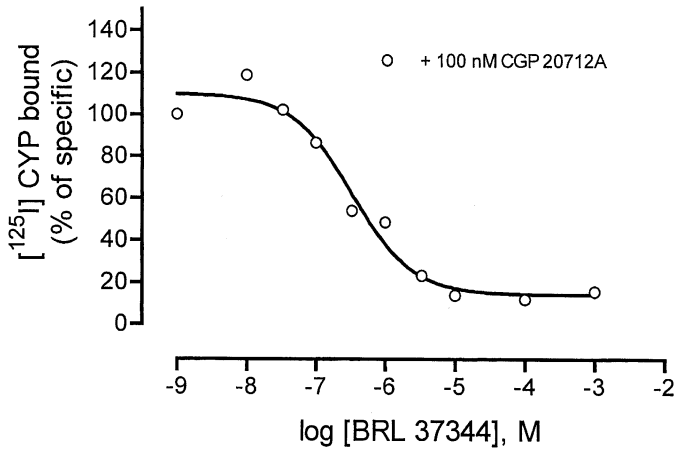

Figure 3. Competitive displacement of specific [^{125}I]CYP binding by BRL 37344 in the presence of 100 nM CGP 20712A. [^{125}I]CYP (~60 pM) was incubated with porcine adipocyte membranes in the presence of increasing concentrations of BRL 37344, 10 μM Gpp(NH)p, and 100 nM CGP 20712A. Data are representative of two independent experiments.

Discussion

Analysis of βAR subtypes requires subtype-selective ligands, which were identified using cloned βAR in CHO cells (Cao, 1998; Liang and Mills, 2001). In addition, [^{125}I]CYP has a similar affinity for pβ_1AR and pβ_2AR in CHO cells, which should have simplified the analysis. However, in confirming the data observed in CHO cells, we found [^{125}I]CYP to have a sixfold greater affinity for the β_2AR than the β_1AR in porcine adipocytes. The discrepancy observed for [^{125}I]CYP between CHO cells and adipocytes will be discussed later. Our data clearly showed that the β_1AR and β_2AR coexisted in porcine tissues. Estimates of the distribution of the β_1AR and β_2AR are in good agreement with the proportion of βAR subtype mRNA in these tissues (McNeel and Mersmann, 1999). These authors used a ribo-

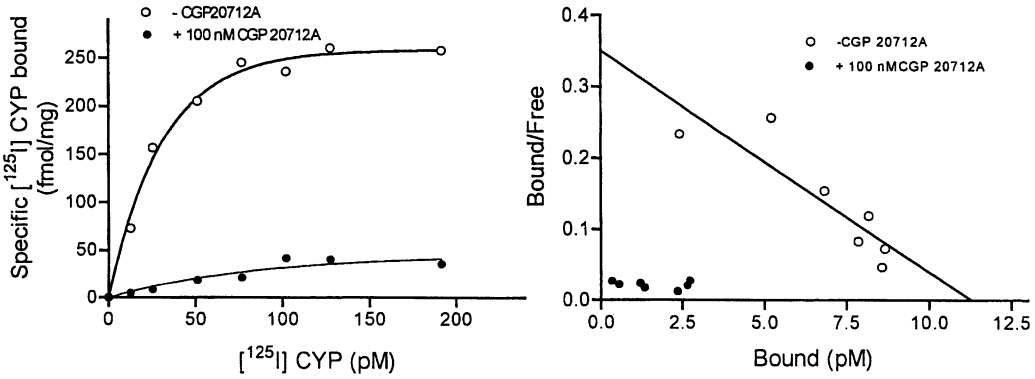

Figure 4. Specific binding of [^{125}I]CYP to porcine adipocyte membranes in the presence and absence of 100 n*M* CGP 20712A. Membranes were incubated with increasing concentrations of [^{125}I]CYP. The inset is the Scatchard plot of the data. The results are representative of three experiments.

nuclease protection assay and found the ratio of β_1AR:β_2AR:β_3AR mRNA to be 73:27:7, 60:39:0.7, 72:28:0.25, 67:33:0.2, and 45:55:0 in adipose tissue, skeletal muscle, heart, lung, and liver, respectively. Few studies have successfully identified multiple βAR subtypes in pig tissues. Coutinho et al. (1992) reported 45% β_1AR and 55% β_2AR in porcine adipocytes, which differs from our estimates. However, these values were determined with only one competitor, ICI 89,406, which showed only a 25-fold selectivity for β_1AR. The close agreement between the current binding data and the mRNA data (McNeel and Mersmann, 1999) strengthens our position that the β_1AR is the predominant βAR in most pig tissues. It should be noted that tissues from the present study came from pigs of different ages (25 to 100 kg), whereas the pigs from McNeel and Mersmann (1999) were about 25 kg. The close agreement between receptor binding and mRNA abundance may suggest that the ratio of βAR subtypes changes little with age, but this would need to be confirmed experimentally.

In most species, including the cat (Molenaar et al., 1985), dog (Liang et al., 1985), human (Ihl-Vahl et al., 1996; Stiles et al., 1983), and rat (Kusumoto et al., 1994), heart tissue contains predominantly β_1AR. Our data for the pig are consistent with other species. However, species differences were evident for other tissues. The β_2AR was the predominant subtype (β_2AR = 60 to 70%) in adipocytes from cattle (Sillence and Matthews, 1994) and human (Mauriege et al., 1988; Sano et al., 1993), and in skeletal muscle in cattle (Ijzerman et al., 1984), cat (Minneman et al., 1979), human (Liggett et al., 1988), mouse (Watson-Wright and Wilkinson, 1986), and rat (Williams et al., 1984). The β_2AR predominants in the lung of most mammalian species, including human (Barnes et al., 1980; Bojanic and Nahorski, 1983; Hauck et al., 1990) and rat (Marullo et al., 1989). The proportion of the β_1AR and β_2AR in porcine liver was 50:50. But, the β_2AR was shown to predominate in liver of rat (Bendeck and Noguchi, 1985), rabbit (Kawai and Arinze, 1983), and human (Sano et al., 1993). We likely have not detected the β_3AR in these studies because of the expected lower affinity for [^{125}I]CYP (De Lean et al., 1982), although affinity estimates have not been determined for the porcine β_3AR. The β_3AR mRNA has been detected in pig tissues, and its abundance is low, representing less than 1% of βAR transcripts in skeletal muscle, heart, and lung, and 7% in adipose tissue (McNeel and Mersmann, 1999). Therefore, it is reasonable to conclude that our assay was detecting primarily the β_1AR and β_2AR subtypes.

Table 2. Distribution of β-adrenergic receptor subtypes in pig tissues[a]

Item	Adipocyte (n = 4)	Skeletal muscle (n =3)	Heart (n = 3)	Lung (n = 3)	Liver (n = 3)
β_1AR (%)[b]	81 ± 2x	59 ± 5^{y}	72 ± 11^{x}	58 ± 4^{y}	50 ± 5^{y}
β_2AR (%)[b]	19 ± 2^{x}	41 ± 10^{y}	28 ± 8^{x}	42 ± 4^{y}	50 ± 5^{y}
Bmax[c]	232 ± 45^{x}	47 ± 3^{z}	493 ± 63^{y}	450 ± 41^{y}	24 ± 3^{z}Pet
K_d[f]	17 ± 6^{x}	75 ± 1^{y}	69 ± 37^{y}	38 ± 4^{x}	46 ± 18^{xy}

[a]Parameters were obtained by saturation analysis conducted in the presence and absence of 100 n*M* CGP 20712A. Means within a row without a common superscript are different ($P \leq 0.05$).
[b]Percentage of total βAR.
[c]Density of βAR (fmol/mg membrane protein) [d]Dissociation constant (p*M*) of total βAR for [^{125}I]CYP, calculated from saturation curves done in the absence of CGP 20712A.

Most of the βAR ligands commonly used to increase protein accretion and reduce fat deposition in animals are purported to be β_2AR-selective (e.g., clenbuterol [Cohen et al., 1982] and L644,969 [Convey et al., 1987]). Pigs generally show less response to these βAR ligands (Mills and Mersmann, 1995; NRC, 1994), particularly clenbuterol, which is approximately 100-fold selective for the β_2AR in pigs (Liang and Mills, 2001). The predominance of β_1AR in porcine adipose tissue and skeletal muscle may contribute to the reduced response. These data imply that a β_1AR-selective agonist should provide the greatest potential for modifying growth and carcass composition. It should be pointed out, however, that subtype density and distribution are only part of the story and selective ligands are needed to determine the subtypes that are most favorably linked to desired responses. Furthermore, targeting of the β_1AR may not provide significant physiological selectivity over a nonselective agonist because the β_1AR appears to be the predominant subtype in most pig tissues. An ideal agonist would selectively target the most desirable events (e.g., protein accretion) and leave untouched the less desirable events (e.g., heart rate).

The densities of total βAR and K_d estimates for [^{125}I]CYP in the porcine tissues agree fairly well with data from pigs and other species (compare Tables 2 and 3). The differences were generally not larger than the variation seen between experiments. The one possible exception was pig heart, which had greater βAR density than other species (Table 3). Procedural differences between labs in the preparation of membrane fractions may contribute to some of the variation, but the data clearly indicate a greater density of βAR in tissues from adipose, heart, and lung compared to skeletal muscle and liver. The significant differences in K_d estimates for [^{125}I]CYP between tissues was not related to subtype distribution because differences were observed between tissues with similar distribution patterns (e.g., adipocytes vs heart, skeletal muscle vs lung; Table 2). Overall, tissue differences were small but the consistency suggests that subtle differences exist, which may be related to differences in membrane lipid and protein composition between cells and tissues (Matsuo and Suzuki, 1994; Seeman et al., 1984).

Affinity differences were also observed for the binding of [^{125}I]CYP to β_1AR and β_2AR in adipocyte membranes but, when expressed in CHO cells, affinity values were similar (Table 4). Differences were not large and were in the same range as differences between tissues, which may suggest that interactions between receptor protein and membrane components may contribute to kinetic differences (Matsuo and Suzuki, 1994; Seeman et al., 1984). A comparison of affinity estimates for CGP and BRL between CHO cells and adipocytes is provided (Table 4). Overall, differences between cell types were small and suggest that the estimates of kinetic parameters from the pβAR-CHO cells are applicable to porcine tissues.

Table 3. Summary of binding parameters for β-adrenergic receptors for different species

Item	Bmax, fmol/mg protein	Kd, p*M*[a]	Reference[b]
Adipocytes			
Pig	150–330	30–152	1, 2
Bovine	648	11	33
Rat240	—	4	
Human	160–320	28–116	5, 6
Skeletal muscle			
Pig	30–60	—	2
Bovine	619	8.8	3
Rat	10–75	60–120	7, 8
Human	10	—	9
Heart			
Pig	70–240	—	10, 11
Rat	60–190	170	12
Human	85	21	6
Lung			
Bovine	252–1,000	—	12, 13
Rat	425	—	14
Human	182	21	6
Liver			
Rat	19	—	15
Human	80	18	6

[a]Dissociation constants are for the radioligand [^{125}I]CYP.
[b]References: 1. Mersmann (1996) 2. Spurlock et al. (1993) 3. Sillence and Matthews (1994) 4. Williams et al. (1976) 5. Mauriege et al. (1988) 6. Sano et al. (1993) 7. Williams et al. (1984) 8. Huang et al. (1998) 9. Liggett et al. (1988) 10. Bocklen et al. (1986) 11. Stadler et al. (1990) 12. Kusumoto et al. (1994) 13. Roets and Burvenich (1995) 14. Cloutier et al. (1998) 15. Dickinson and Nahorski (1981) 16. Bendeck and Noguchi (1985).

Implications

The β-adrenergic receptors represent a family of membrane proteins that are targets for growth and composition modification in livestock. The β_1-adrenergic receptor is the predominant subtype in the major porcine tissues, particularly adipose tissue. These data imply that the most efficacious ligands for modifying growth (particularly adipose tissue accretion) should effectively signal through the β_1-adrenergic receptor. However, it remains to be determined whether the β-adrenergic receptor subtypes have redundant roles in regulating growth processes in each cell.

Table 4. Comparison of affinity estimates (p*M*) for cloned and native pig β-adrenergic receptors[a]

	β_1AR		β_2AR	
Ligand	Cloned βAR[a]	Adipocytes[b]	Cloned βAR[a]	Adipocytes[b]
[^{125}I]CYP	19	57–64	14.5	3.2–11.2
CGP 20712a	.5	2.6	404	418
BRL 37344	9805	16,050	122	5.3 (139)[2]

[a]Data are from cloned βAR expressed in Chinese hamster ovary cells (Cao, 1998; Liang and Mills, 2001).
[b]Data are from Table 1.

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