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ORIGINAL PAPER

Effects of dexamethasone on the expression of β_1-, β_2- and β_3-adrenoceptor mRNAs in skeletal and left ventricle muscles in rats

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Abstract Glucocorticoids are known to increase the density and mRNA levels of β-adrenoceptors (β-AR) via the glucocorticoid receptor (GR) in many tissues. However, the effects of these changes in the skeletal and cardiac muscles remain relatively unknown. We have investigated the effects of dexamethasone on the expression of the β_1-, β_2-, and β_3-AR mRNAs and GR mRNA in fast-twitch fiber-rich extensor digitorum longus (EDL), slow-twitch fiber-rich soleus (SOL), and left ventricle (LV) muscles by real-time quantitative RT-PCR. Male rats were divided into a dexamethasone group and control group. The weight, RNA concentration, and total RNA content of EDL muscle were 0.76-, 0.85-, and 0.65-fold lower, respectively, in the dexamethasone group than in the control group. The weight, RNA concentration, and total RNA content of SOL muscle were 0.92-, 0.87-, and 0.81-fold lower, respectively, in the dexamethasone group than in the control group; these differences were significant. However, the weight/body weight and total RNA content/body weight of LV muscle were 1.38- and 1.39-fold higher, respectively, in the dexamethasone group than in the control group, respectively; these differences were also significant. Dexamethasone significantly decreased GR mRNA expression in EDL muscle without changing the expression of the β_1-, β_2-, and β_3-AR mRNAs. However, dexamethasone significantly decreased the expressions of β_2-AR and GR mRNAs in SOL muscle and significantly increased β_1-AR mRNA expression in LV muscle—without changing GR mRNA expression. These results suggest that the effects of dexamethasone on the expression of β_1- and β_2-AR mRNAs and muscle mass depend on the muscle contractile and/or constructive types.

Keywords β-Adrenoceptor mRNA · Glucocorticoid receptor mRNA · Muscle mass · Skeletal and left ventricle muscles · Synthesized glucocorticoid dexamethasone

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Introduction

Many substances (i.e., β_2-agonists, androgenic anabolic steroids, growth hormones, erythropoietins, fibroblast growth factors 1, 2, 4, 6, and 9, mechano-growth factors, and insulin-like growth factor-1) have been used as doping drugs for the improvement of athletic performance, with effects such as increasing muscle strength, muscle power, and endurance capacity [1–3]. β_2-Agonists (i.e., clenbuterol, salbutamol, metaproterenol, fenoterol, and clorpreneline) and anabolic androgenic steroids (i.e., 19-norandrogen, 19-norandrostendion, stanozolol, methandienone, and nandrolone), for example are known to heighten muscle strength

and muscle power [4–9] and have, therefore, been used as a non-steroidal anabolic doping drug by athletes to increase skeletal muscle mass and decrease body fat. In particular, according to the recent World Anti-Doping Agency (WADA) documents, the β_2-agonist, clenbuterol [4-amino-α (*t*-butyl-amino) methyl-3, 5-dichlorobenzyl alcohol] was the fifth most common contravention (53 cases) of anabolic drugs in 2006 [10].

It is generally accepted that the β_2-agonist clenbuterol induces muscle hypertrophy by stimulating protein synthesis and inhibiting protein degradation [11]. β_2-Agonists are also known to induce skeletal muscle hypertrophy via the β_2-adrenoceptor (β_2-AR), and the β_2-AR mediates the effects of catecholamine in many physiological responses [11]. However, the precise mechanism of the increased actions of clenbuterol-induced muscle power and muscle mass in skeletal muscles is still unknown.

We recently reported that the effects of clenbuterol [dose = 1.0 mg/kg body weight per day for 10 days, subcutaneously (s.c.)] on the expression of β_1- and β_2-AR mRNAs in fast-twitch fiber-rich extensor digitorum longus (EDL), slow-twitch fiber-rich soleus (SOL) and left ventricle (LV) muscles [12]. In rats, skeletal muscles are composed of typical fast-twitch fiber-rich muscles, such as the EDL muscle and typical slow-twitch fiber-rich muscles, such as the SOL muscle [7, 13, 14]. Each skeletal muscle is different in terms of the relative composition of these muscle fibers, and the muscle fibers themselves are different in terms of their velocity of contraction, metabolic properties, and β-AR distributions [4, 7, 13–15]. The administration of clenbuterol was found to significantly increase the weight, RNA concentration, and total RNA content of EDL muscle [12], but this drug had no effect on these parameters in SOL and LV muscles [12]. In the same study, the administration of clenbuterol was found to significantly decrease the expression of β_1-AR mRNA in LV muscle [12], significantly decrease the expression of β_2-AR mRNA in EDL and LV muscles [12], and to have no effect on β_2-AR mRNA expression in SOL muscle [12]. On the contrary, Jensen et al. [16, 17] reported that of the total β-ARs in skeletal muscles, about 80–95% were of the β_2-AR subtype, with the slow-twitch fiber-rich SOL muscle containing relatively more β_2-AR than the fast-twitch fiber-rich EDL muscle. These results suggest that the effects of clenbuterol on the expression of β_1- and β_2-AR mRNAs and muscle hypertrophy depend on the types of muscle fiber and not on the number of β-ARs [12].

Alternatively, glucocorticoids are known to decrease the rate of protein synthesis and increase the rate of protein degradation, leading to muscle atrophy [18–20]. Glucocorticoids increase the expression and activity of the ubiquitin–proteasome pathway that play an important role in the major proteolytic mechanism of muscle atrophy [21]. In particular, the ubiquitin–proteasome system of muscle proteolysis is considered to play a major role in the catabolic action of glucocorticoids [22]. It is well known that EDL muscle shows more glucocorticoid-induced muscle atrophy than SOL muscle [23].

Cornett et al. [24] reported that glucocorticoids increased β_2-AR mRNA by acting as a glucocorticoid response element (GRE) on β_2-AR gene via the glucocorticoid receptor (GR). Huang et al. [25, 26] also showed that the administration of dexamethasone for 5 or 10 days to rats increased β_2-AR density in lung tissue. However, the effects of dexamethasone on the regulation of β_2-AR and GR expression levels in the skeletal and cardiac muscles have not been elucidated. Here, we report on our investigation of the effects of dexamethasone on the expression of β_1-, β_2-, and β_3-AR and GR mRNA levels in EDL, SOL, and LV muscles in rats.

Materials and methods

Experimental procedures and animal care

The protocol of our study is shown in Fig. 1. During the experimental period, synthesized glucocorticoid

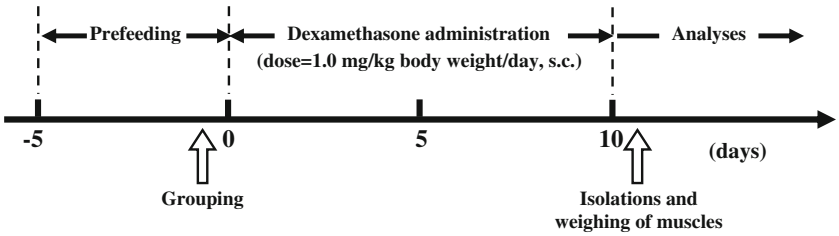

Fig. 1 Experimental protocol. After a 5-day adaptation period, the rats were divided into the dexamethasone group ($n = 16$) and the control group ($n = 15$). Dexamethasone (dose = 1.0 mg/kg body weight per day) was administered from the cervical portion of the back via a subcutaneous (*s.c.*) injection (0900–0930 hours) for 10 consecutive days. In control group rats, an equivalent volume of 0.9% NaCl solution was administered in the same manner. Muscles were isolated and weighed on the day after the final day of the administration of dexamethasone

dexamethasone was administered to rats for 10 days (dose = 1.0 mg/kg body weight per day). The EDL, SOL, and LV muscles were isolated and weighed on the day after the final day of the administration of dexamethasone to clarify the effects of dexamethasone on the expression of β_1-, β_2-, β_3-AR and GR mRNAs in EDL, SOL and LV muscles [12].

Male 7-week-old Sprague–Dawley rats (CLEA Japan, Tokyo) were pre-fed for 5 days to allow adaptation to their new environment [12, 27–29]. The rats were maintained at a controlled temperature (23–25°C) and relative humidity (50–60%), with fixed light–dark cycles [0900–2100 hours (light) and 2100–0900 hours (dark)] [12, 27, 28]. Animal foods (CE-2 cubic type, CLEA Japan) were given to each rat under pair feeding, and distilled water was given ad libitum [12, 27, 28]. All rats were weighed daily during the experimental periods. After the adaptation period, the rats were randomly divided into two groups: a dexamethasone group [$n = 16$, initial body weight 276 ± 3 g, mean ± standard error of the mean (SEM)] group and a control group ($n = 15$, initial body weight 277 ± 3 g, mean ± SEM).

All experimental and animal care procedures were approved by the Committee on Animal Care Use at Waseda University and followed the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences established by the Physiological Society of Japan [12, 27–30] and the American Physiological Society Animal Care Guidelines. We performed procedures with the least possible pain or discomfort to the rats [12, 27–29].

Administration of dexamethasone to rats

Dexamethasone 21-phosphate (Sigma, St. Louis, MO) was dissolved in a 0.9% NaCl solution as a vehicle to obtain a dexamethasone concentration of 0.1%. In the dexamethasone group, dexamethasone (dose 1.0 mg/kg body weight per day) was administered to the cervical portion of the back via a subcutaneous (s.c.) injection (0900–0930 hours) for 10 days [12]. In the control group, an equivalent volume of 0.9% NaCl solution was administered in the same manner [12, 27–29].

Sample storage

Isolated and weighed skeletal muscle was cut the both ends and rapidly frozen in liquid nitrogen to stabilize RNA. In addition, residual blood in the isolated heart muscle was removed by washing with an autoclaved 0.9% NaCl solution, and then the heart muscles were separated into LV and the other sections [12]. The LV was then weighed and rapidly frozen in liquid nitrogen to stabilize the RNA, and the samples were stored at −80°C until they could be used for RNA extraction.

Analyses of mRNA expression by real-time quantitative reverse transcription-PCR

Real-time quantitative reverse transcription (RT)-PCR was used to quantify the expression levels of β_1-, β_2-, β_3-AR and GR mRNAs. Total RNA in muscles was extracted from stored muscle samples using a TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. The total RNA concentration was determined by measuring absorbance at 260 and 280 nm (U-3310 Spectrophotometer; Hitachi, Tokyo) according to our routine method [31].

The extracted total RNA was subjected to single-stranded cDNA synthesis using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. In real-time quantitative RT-PCR, synthesized cDNA is added to a power SYBR green PCR master mix (Applied Biosystems) containing 50 μ*M* PCR primers (forward and reverse) [12, 31].

The primer oligonucleotide sequences used for real-time quantitative RT-PCR were:

β_1-AR: 5′-CTG CTA CAA CGA CCC CAA GTG-3′ (forward) and 5′-AAC ACC CGG AGG TAC ACG AA-3′ (reverse);
β_2-AR: 5′-GAG CCA CAC GGG AAT GAC A-3′ (forward) and 5′-CCA GGA CGA TAA CCG ACA TGA-3′ (reverse);
β_3-AR: 5′-GAG GCA ACC TGC TGG TAA TCA C-3′ (forward) and 5′-ACG AGG AGT CCC ACT ACC AAG TC-3′ (reverse);
GR: 5′-TAC CAC AGC TCA CCC CTA CC-3′ (forward) and 5′-AGC AGG GTC ATT TGG TCA TC-3′ (reverse).

TATA-box binding protein (Tbp) was used as the reference gene [32–34]. Amplification was performed using an ABI PRISM 7000 Sequence Detection System (Applied Biosystems). β_1-, β_2-, and β_3-AR and GR mRNA levels were normalized using the threshold cycle (Ct) method in accordance with the manufacturer's protocol and were expressed as relative ratios to Tbp mRNA levels [12, 31].

Statistical analyses

Experimental data are presented as the mean ± SEM. The differences between two groups were tested by a Student's *t* test and considered to be significant when P was <0.05.

Results

The body weight, body weight gain, total food intake, and total water intake during the experimental period

Although there were no significant differences in the total food intake and total water intake between both groups, the body weight in the dexamethasone group was 0.76-fold ($P < 0.001$) lower than that in the control group (Table 1). The body weight gains were estimated as 16 ± 3 g in the control group and −52 ± 3 g in the dexamethasone group (Table 1).

Table 1 Effects of dexamethasone on the body weight, body weight gain, total food intake and total water intake in rats

Parameters	Group	
	Control ($n = 15$)	Dexamethasone ($n = 16$)
Body weight (g)	288 ± 3	220 ± 5***
Body weight gain (g)	16 ± 3	−52 ± 3***
Total food intake (g)	199 ± 0.1	189 ± 5.3
Total water intake (g)	464 ± 24	475 ± 26

Values are given as the mean ± standard error of the mean (SEM)

*** $P < 0.001$ (vs. control group)

The weights, RNA concentrations, total RNA contents, and total RNA content/body weight of skeletal and left ventricle muscles

As shown in Table 2, the weight of the EDL muscle in the dexamethasone group was 0.76-fold ($P < 0.001$) lower than that in the control group. RNA concentration, total RNA content, and total RNA content/body weight of EDL muscle were 0.85- ($P < 0.01$), 0.65- ($P < 0.001$), and 0.84-fold ($P < 0.05$) lower in the dexamethasone group than in the control group, respectively. The weight, RNA concentration, and total RNA content of the SOL muscle were 0.92- ($P < 0.05$), 0.87- ($P < 0.01$), and 0.81-fold ($P < 0.001$) lower in the dexamethasone group than in the control group, respectively. The weight/body weight of SOL muscle was 1.21-fold ($P < 0.001$) higher in the dexamethasone group than in the control group. No significant differences in terms of the weight, RNA concentration, and total RNA content of LV muscle were observed between both groups. However, the weight/body weight and total RNA content/body weight of LV muscle were

Table 2 Effects of dexamethasone on the body weight and the weight, RNA concentration, total RNA content, and total RNA content/body weight in EDL, SOL, and LV muscles

Parameters	Group		
	Control ($n = 15$)	Dexamethasone ($n = 16$)	Dexamethasone/control[a]
EDL			
Weight (mg)	152 ± 4	115 ± 2***	0.76
Weight (mg)/body weight (g)	0.53 ± 0.01	0.52 ± 0.01	0.99
RNA concentration (μg/g tissue)	713 ± 26	604 ± 19**	0.85
Total RNA content (μg)	105 ± 3	68 ± 4***	0.65
Total RNA content (μg)/body weight (g)	0.37 ± 0.01	0.31 ± 0.02*	0.84
SOL			
Weight (mg)	114 ± 3	105 ± 2*	0.92
Weight (mg)/body weight (g)	0.40 ± 0.01	0.48 ± 0.01***	1.21
RNA concentration (μg/g tissue)	1225 ± 37	1061 ± 37**	0.87
Total RNA content (μg)	138 ± 4	112 ± 6***	0.81
Total RNA content (μg)/body weight (g)	0.48 ± 0.02	0.51 ± 0.02	1.06
LV			
Weight (mg)	467 ± 30	485 ± 20	1.04
Weight (mg)/body weight (g)	1.63 ± 0.11	2.25 ± 0.13***	1.38
RNA concentration (μg/g tissue)	830 ± 38	845 ± 27	1.02
Total RNA content (μg)	395 ± 38	414 ± 26	1.05
Total RNA content (μg)/body weight (g)	1.38 ± 0.1	1.92 ± 0.2*	1.39

Values are given as the mean ± SEM

EDL Extensor digitorum longus, *SOL* soleus muscle, *LV* left ventricle muscle

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (vs. control group)

[a] Dexamethasone/control, The relative ratio of the dexamethasone group to the control group

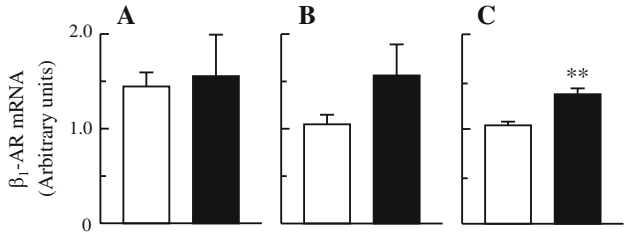

Fig. 2 Effects of dexamethasone on the expression of β_1-adrenoceptor (β_1-*AR*) mRNA in extensor digitorum longus (EDL, **a**), soleus muscle (SOL, **b**), and left ventricle (LV, **c**) muscle. The values are given as the mean ± SEM. *Open bar* control group ($n = 15$), *closed bar* dexamethasone group ($n = 16$). $^{**}P < 0.01$ (vs. control group)

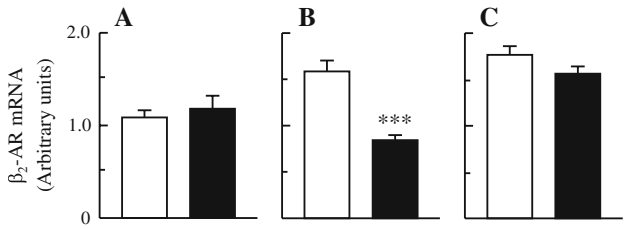

Fig. 3 Effects of dexamethasone on the expression of β_2-*AR* mRNA in EDL (**a**), SOL (**b**), and LV (**c**) muscles. Values are given as the mean ± SEM. *Open bar* control group ($n = 15$), *closed bar* dexamethasone group ($n = 16$). $^{***}P < 0.001$ (vs. control group)

1.38- ($P < 0.001$) and 1.39-fold ($P < 0.05$) higher in the dexamethasone group than in the control group, respectively.

Expression of β_1-AR mRNA in muscles

Figure 2 shows the effects of dexamethasone on β_1-AR mRNA expression in EDL, SOL and LV muscles. There were no significant differences in the expression of β_1-AR mRNA in EDL (Fig. 2a) and SOL (Fig. 2b) muscles between both groups. However, β_1-AR mRNA expression in LV muscle was 1.31-fold ($P < 0.05$) higher in the dexamethasone group than in the control group (Fig. 2c).

Expression of β_2-AR mRNA in muscles

As shown in Fig. 3, the expression of β_2-AR mRNA in SOL was 0.53-fold ($P < 0.001$) lower in the dexamethasone group than in the control group (Fig. 3b). However, there were no significant differences in terms of β_2-AR mRNA expression in the EDL (Fig. 3a) and LV (Fig. 3c) muscles between both groups.

Expression of β_3-AR mRNA in muscles

As shown in Fig. 4, there were no significant differences in β_3-AR mRNA expression in the EDL (Fig. 4a), SOL (Fig. 4b), and LV (Fig. 4c) muscles between both groups.

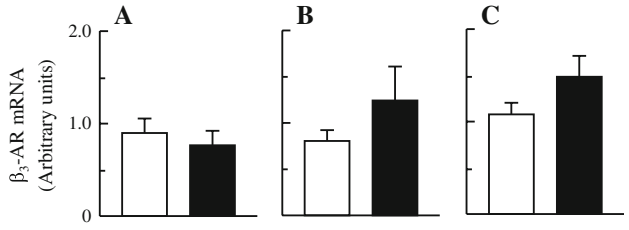

Fig. 4 Effects of dexamethasone on the expression of β_3-*AR* mRNA in EDL (**a**), SOL (**b**), and LV (**c**) muscles. The values are given as the mean ± SEM. *Open bar* control group ($n = 15$), *closed bar* dexamethasone group ($n = 16$)

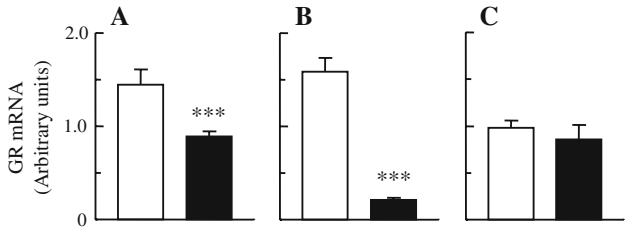

Fig. 5 Effects of dexamethasone on the expression of glucocorticoid receptor (*GR*) mRNA in EDL (**a**), SOL (**b**), and LV (**c**) muscles. The values are given as the mean ± SEM. *Open bar* control group ($n = 15$), *closed bar* dexamethasone group ($n = 16$). $^{***}P < 0.001$ (vs. control group)

Expression of GR mRNA in muscles

Figure 5 shows the effects of dexamethasone on GR mRNA expression in EDL, SOL, and LV muscles. The GR mRNA expression in the EDL (Fig. 5a) and SOL (Fig. 5b) muscles was 0.61- ($P < 0.001$) and 0.13-fold ($P < 0.001$) lower in the dexamethasone group than in the control group, respectively. However, there were no significant differences in terms of GR mRNA expression in the LV muscle between both groups (Fig. 5c).

Discussion

Table 3 presents a summary of the results. A number of observations were noted. (1) Body weight was significantly lower in the dexamethasone group than in control group (Table 1); the weight, RNA concentration, total RNA content, and total RNA content/body weight of EDL muscle were significantly lower in the dexamethasone group than in the control group (Table 2); the weight, RNA concentration, and total RNA content of SOL muscle were significantly lower in the dexamethasone group than in the control group (Table 2); the weight/body weight and total RNA content/body weight of LV muscle were significantly higher in the dexamethasone group than in the control group (Table 2). (2) The administration of dexamethasone

Table 3 Summary of effects of dexamethasone on the weight, weight/body weight, RNA concentration, total RNA content, total RNA content/body weight, and expressions of β_1-, β_2-, and β_3-AR and GR mRNA of EDL, SOL and LV muscles

Muscles	Weight	Weight/body weight	RNA concentration	Total RNA content	Total RNA content/body weight	β_1-AR mRNA expression	β_2-AR mRNA expression	β_3-AR mRNA expression	GR mRNA expression
EDL	↓ (0.76)	n.s.	↓ (0.85)	↓ (0.65)	↓ (0.84)	n.s.	n.s.	n.s.	↓ (0.61)
SOL	↓ (0.92)	↑ (1.21)	↓ (0.87)	↓ (0.81)	n.s.	n.s.	↓ (0.53)	n.s.	↓ (0.13)
LV	n.s.	↑ (1.38)	n.s.	n.s.	↑ (1.39)	↑ (1.31)	n.s.	n.s.	n.s.

n.s. Not significant between both groups, *AR* adrenoceptor, *GR* glucocorticoid receptor

Value in parenthesis is the relative ratio of the dexamethasone group to the control group; upwards-pointing arrow, value is significantly higher in the dexamethasone group than in the control group; downwards-pointing arrow, value is significantly lower in the dexamethasone group than in the control group

significantly increased β_1-AR mRNA expression in the LV muscle. However, there were no significant changes in the expression of β_1-AR mRNA in EDL and SOL muscles between both groups (Fig. 2). (3) The administration of dexamethasone significantly decreased the expression of β_2-AR mRNA in SOL muscle. However, there were no significant changes in the expression of β_2-AR mRNA in EDL and LV muscles between both groups (Fig. 3). (4) The administration of dexamethasone had no effects on β_3-AR mRNA expression in EDL, SOL, and LV muscles (Fig. 4). (5) The administration of dexamethasone significantly decreased GR mRNA expression in EDL and SOL muscles; however, there was no significant change in GR mRNA expression in LV muscle between both groups (Fig. 5). These results suggest that the effects of dexamethasone on the expression of β_1- and β_2-AR mRNAs and muscle mass depend on muscle contractile and/or constructive types.

Skeletal muscle weights

In agreement with our previous findings [35], our results clearly confirm that in our study model dexamethasone decreased body weight and body weight gain (Table 1). We also found that dexamethasone significantly decreased the weight of the EDL and SOL muscles (Table 2). However, we also demonstrated that dexamethasone increased the relative weight/body weight of the SOL muscle without changing the relative weight of the EDL muscle (Table 2). Takes together, these findings indicate that the degree of muscle atrophy is relatively smaller in the SOL muscle than in the EDL muscle. Livingstone et al. [23] also reported that dexamethasone-induced muscle atrophy was relatively higher in EDL muscle than in SOL muscle. Dekhuijzen et al. [36] and Fournier et al. [37] also reported that glucocorticoid have been shown to cause the atrophy of fast-twitch or type II muscle fibers (particularly IIx and IIb) with less or no impact observed in type I fibers. Our results show that dexamethasone-induced actions on total RNA content and total RNA content/body weight are relatively larger in the fast-twitch fiber-rich EDL muscle than in slow-twitch fiber-rich SOL muscle (Table 2). Therefore, these results and those of previous studies suggest that dexamethasone decreases the synthesis rate of muscle protein and/or increases the degradation rate of muscle protein more specifically in fast-twitch type II fiber-rich muscle, such as EDL muscle, than in slow-twitch type I fiber-rich muscle, such as SOL muscle. The precise mechanism of such fiber type specificities, however, is not yet known.

Expression of β_1-, β_2-, and β_3-AR and GR mRNAs in skeletal muscles

In the study reported here, we have clearly demonstrated that dexamethasone significantly decreased the expression of β_2-AR mRNA in SOL muscle (Fig. 3b). However, no significant effect of dexamethasone on the expression of β_2-AR mRNA in the EDL muscle was observed (Fig. 3a). We also showed that dexamethasone significantly decreased the expression of GR mRNA in the EDL (Fig. 5a) and SOL (Fig. 5b) muscles. These results together suggest that the different responses of dexamethasone on β_2-AR mRNA expression between fast-twitch fiber-rich EDL muscle and slow-twitch fiber-rich SOL muscle are associated with the number and distribution of β_2-AR molecules and/or the degree of reduction in GR mRNA expression in both skeletal muscles.

Jensen et al. [16, 17] reported that about 80–95% of total β-ARs in skeletal muscles were of the β_2-AR subtype and that the density of β_2-AR was about twofold higher in the slow-twitch fiber-rich SOL muscle than in the fast-twitch

fiber-rich EDL muscle. These distributions of the β-AR subtypes may explain the absence of significant effects between the expression levels of β_1- and β_3-AR mRNAs in the EDL and SOL muscles between both groups. They may also indicate the density-dependent decrease of β_2-AR mRNA expression in SOL muscle. However, it has been reported that the β_2-agonist clenbuterol decreased β_2-AR mRNA expression in EDL muscle in a β_2-AR density-independent manner [12]. Therefore, the effects of dexamethasone and clenbuterol on the β_2-AR mRNA expression of fast- and slow-twitch muscles are still uncertain in terms of β_2-AR density dependence/independence.

However, Cornett et al. [24] reported that glucocorticoids increased β_2-AR mRNA by acting as a GRE on the β_2-AR gene via the GR. However, Cleasby et al. [38] reported that dexamethasone decreased GR mRNA expression in the SOL muscle without changing the expression of GR mRNA in EDL muscle. In fact, although dexamethasone significantly decreased the expression of GR mRNA expression in the EDL (Fig. 5a) and SOL (Fig. 5b) muscles, the degree of decline of GR mRNA expression was much greater in SOL muscle than in EDL muscle. These results suggest that the reduction of β_2-AR mRNA expression in the SOL muscle is related to the relatively larger reduction in GR mRNA in the SOL muscle.

Expression of β_1-, β_2-, and β_3-AR and GR mRNAs in LV muscle

Our results clearly show that dexamethasone significantly increased the expression of β_1-AR mRNA in LV muscle, without changing the expression of β_2-AR, β_3-AR, and GR mRNAs (Figs. 2c, 3c, 4c, 5c) and also increased the body weight and total RNA content/body weight of LV muscle (Table 2).

Although β_1-, β_2-, and β_3-AR are expressed in cardiomyocytes, β_1-AR is known to be the predominant receptor and have positive inotropic effects [39]. These findings suggest that the hypertrophy of cardiac muscle and the increase of contraction strength cause the upregulation of β_1-AR mRNA expression in LV muscle. Tseng et al. [40] reported that a complex glucocorticoid regulatory unit has been discovered in the promoter region of the β_1-AR gene. Although it is unclear how much this unit contributes to the promotion of β_1-AR transcriptional regulation, the expression of β_1-AR mRNA in LV muscle may be upregulated via a stimulation of the unit by dexamethasone.

We also found that there was no significant difference in the expression of β_2-AR (Fig. 3c) and GR mRNAs (Fig. 5c) in LV muscle between the dexamethasone and control groups. However, Dangel et al. [41] reported that dexamethasone increased β_2-AR mRNA level via GRE, although the β_1-AR mRNA level was not changed during treatment of dexamethasone for 24 h in cardiocyte-derived cell line H9c2. Mysliveček et al. [42] also reported that the administration of the glucocorticoid hydrocortisone (50–100 mg/kg body weight per day for 9 days) to adult male Wister rats significantly increased the density of β_1-AR in atria without changing that in ventricles and also significantly increased the density of β_2-AR in both the atria and ventricles. Furthermore, Kalinyak et al. [43] reported that dexamethasone decreased GR mRNA expression in cardiac muscle. The different effects of glucocorticoids on β_2-AR and GR mRNA expressions in LV muscle between our study and previous ones are paradoxical.

Both the results of the study reported here and those of an earlier study by our group [12] reveal that the effects of dexamethasone and clenbuterol on the expression of β_1-AR mRNA in LV muscle are upregulatory (Fig. 2c) and downregulatory, respectively. However, the precise mechanisms of these different responses in β_1-AR mRNA expression in LV muscle are still unknown. More elaborated studies are necessary to clarify the mechanism of the different responses of dexamethasone and clenbuterol on β_1-AR mRNA expression in LV muscle.

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