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Do sex steroids regulate glutamine synthesis with age?

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Verdier, Lionel, Yves Boirie, Sebastien Van Drieessche, Michelle Mignon, Rene-Jean Begue, and Dominique Meynial-Denis. Do sex steroids regulate glutamine synthesis with age? *Am J Physiol Endocrinol Metab* 282: E215–E221, 2001; 10.1152/ajpendo.00117.2001.—Glutamine synthetase, a key enzyme in the production of glutamine, is known to be induced by glucocorticoids and preserved in skeletal muscle during aging, but the effect of other steroids, such as sex steroids (progesterone, estradiol), is unknown in vivo. The aim of this study was to determine whether progesterone or estradiol plays a role in the regulation of glutamine synthetase (GS) with aging. The effects of glucocorticoids and sex steroids on muscle GS activity and mRNA expression were measured in adult (6–8 mo; $n = 7$ in each group) and aged (26 mo; $n = 10$ in each group) female Wistar rats after adrenalectomy (ADX), ovariectomy (OV), or both (ADXOV) and were compared with those in sham-operated (Sham) control rats. In tibialis anterior muscle, ADX noticeably decreased both GS activity and expression irrespective of age (50–60%; $P < 0.05$), whereas OV had no effect at either age. Progesterone and estradiol replacement had no effect on the recovery of muscle GS response in either ADX or OV rats, regardless of age. In contrast, heart GS activity was decreased by ADX in aged animals only. These results suggest that the reproductive endocrine status of female rats does not affect muscle GS activity either in muscle or in heart, in young or aged animals, and that the heart GS response to steroids may be differently regulated in aged rats.

rat; muscle; heart; glucocorticoids

IN BOTH HUMANS AND RODENTS, alterations in muscle protein synthesis occur during the aging process, leading to a loss of muscle protein, or sarcopenia (9, 23, 26). This age-related loss of muscle mass is associated with an increased risk of morbidity, mortality, and disability in old age, but the mechanisms by which this occurs are not fully understood (4). In addition to proteins, muscle is a large reservoir of free amino acids, among which glutamine (Gln) is the most abundant (11, 29). Skeletal muscle loss and protein depletion may lead to Gln deficiency, which could in turn contribute to the high susceptibility of elderly people to catabolic situa-

tions (34). Even under normal conditions, Gln depletion may play a prominent role in aging. Indeed, it is well known that the immune function declines with aging (12). Because Gln is a preferred source of energy for the immune system (24), and because muscle synthesizes, stores, and releases Gln (7), muscle wasting may contribute to the decline of immune function associated with aging. However, previous studies demonstrated that, in the basal state, endogenous whole body rate of appearance of Gln was similar in young and elderly volunteers (22). Moreover, plasma and muscle Gln levels are normal in aged rats (20, 21). Although these results appear as a paradox, it may be explained by the ability of muscle to maintain a high capacity for Gln synthesis, even if muscle atrophy is suspected. For this reason, the regulation of Gln metabolism in aged muscle deserves further investigation under basal conditions.

Glutamine synthetase (GS), which catalyzes de novo Gln synthesis from its precursors glutamate and ammonia, is a crucial enzyme for Gln homeostasis (13). Indeed, in catabolic states (for review, see Refs. 3, 11) when Gln is exported in large amounts from muscle and becomes a conditionally indispensable amino acid, the maintenance of the muscle Gln pool depends on continuous de novo Gln synthesis. We have already examined GS in aged rats in a catabolic situation (glucocorticoid excess) (20). We reported that, regardless of the age of animals, glucocorticoids are a major modulator of GS. This is supported by the interaction of the glucocorticoid receptor complex with a glucocorticoid-responsive element in the GS gene (16, 28, 30). By contrast, to our knowledge, the action of endogenous glucocorticoids and other hormones like sex steroids on Gln metabolism has not been determined in muscle in vivo.

The role of progesterone and estradiol in the regulation of GS has been investigated in mouse fibroblasts (10) and in L6 muscle cells (19). In these cells, progesterone displays some agonist activity in terms of induc-

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tion of GS (an increase of ~2-fold instead of 8- to 10-fold by a synthetic glucocorticoid compared with control conditions), but estradiol is devoid of stimulatory activity. The effect of progesterone may be due to its interaction with glucocorticoid receptors (19). By contrast, to our knowledge, no data on GS regulation by sex steroids *in vivo* have ever been reported in skeletal muscle either in adult or in aged animals. Moreover, whereas a role for estradiol and progesterone in the modulation of fat-free mass (skeletal muscle in major part) has been demonstrated in growing female rats (32), there are no data in adult and aged rats. We therefore chose to analyze the effect of sex steroid depletion on GS activity and GS mRNA expression in skeletal muscle from adult and aged female rats. For this aim, we studied GS responsiveness in tibialis anterior (mixed) and soleus (oxidative) muscles and in heart obtained from female rats either after ovariectomy, after adrenalectomy, or after both ovariectomy and adrenalectomy. In further experiments, we tested the effect of hormone replacement after total deprivation of both glucocorticoids and sex steroids.

MATERIALS AND METHODS

Materials

Progesterone and 17 β -estradiol were obtained from Sigma Chemical (St. Louis, MO). [14 C]glutamine (262 mCi/mmol) was purchased from Amersham (Buckinghamshire, UK). The cDNA probe encoding the rat GS (no. 63067) and the mouse rRNA 18S probe (no. 63178) were purchased from the American Type Culture Collection (Rockville, MD).

Animals and Surgical Protocol

All animals and surgical experimental procedures were used in accordance with recommendations from the Institutional Ethics Committee (University of Clermont-Ferrand). Adult (6- to 8-mo) female Wistar rats were purchased from IFFA-CREDO (L'Arbresle, France) and were nonpregnant. Animals were housed in separate cages in a room with a 12:12-h light-dark cycle (lights on at 8:00 AM) at 22°C. The rats were fed with Purina rat chow and water *ad libitum*. Aged rats were housed in the animal facilities until they reached 26 mo of age. At the beginning of experiments, both adult and aged rats weighed between 300 and 400 g.

Experiment 1: effect of adrenalectomy or ovariectomy or both adrenalectomy and ovariectomy on GS activity. Adult and aged rats were either sham-operated (Sham), adrenalectomized (ADX), ovariectomized (OV), or both adrenalectomized and ovariectomized (ADXOV) under anesthesia with 2 ml/kg body wt of a mixture of Imalgène 500 and Vetranquil 0.5% (vol/vol 3:1, ip). Each group consisted of 7 adult rats or 10 aged rats. Animals received normal feeding and 0.9% NaCl solution (only for rats undergoing ADX) and glucose in addition to water *ad libitum* (to facilitate the recovery of animals). Animals were studied 7 days after surgery. Body weight and food consumption were recorded daily during the 7-day period of recovery. Rats were anesthetized with pentobarbital sodium (45 μ g/g body wt ip) before blood sampling and excision of the tibialis anterior muscle, soleus muscle, and heart. The tissues were weighed and quickly frozen in liquid nitrogen.

Experiment 2: hormone replacement experiment. To clearly examine the role of glucocorticoids and sex steroids in the

basal response of the GS enzyme, we suppressed the physiological production of these hormones and then added each hormone in a separate experiment. For this, five groups of adult rats (6–8 mo) were surgically ADXOV ($n = 4$ –6 rats in each group). On *days 2* through *6* after surgery (to allow clearance of circulating glucocorticoids and/or sex steroids), rats were subcutaneously injected with 17 β -estradiol (E_2 ; 10 μ g/kg body wt $^{-1}$ ·h $^{-1}$ on *days 2, 4, and 6*), progesterone (P; 140 μ g/kg body wt $^{-1}$ ·48 h $^{-1}$ on *days 2, 4, and 6*), or dexamethasone (DEX; 0.1 mg/kg body wt by a single injection on *day 6*). E_2 and P were diluted in a minimum volume of ethanol and then added with sesame oil (to allow slow diffusion of hormones), as previously reported (8); DEX was diluted in water.

For P or E_2 experiments, two control groups were used. A group of ADXOV rats and a group of Sham rats were injected subcutaneously with 50 μ l/100 g body wt of sesame oil containing 0.5% ethanol over the same treatment period to 1) assess the activity of GS in the absence of either glucocorticoids or sex steroids and 2) ensure that solvent had no effect on GS activity. The control group for the DEX experiment consisted of ADXOV rats receiving a subcutaneous injection of water (vehicle) on *day 6*. For aged rats (26 mo), only two groups underwent surgical ADXOV and were subsequently injected with either E_2 (10 μ g/kg body wt $^{-1}$ ·48 h $^{-1}$ between *day 2* and *day 6* after surgery) or vehicle. On *day 7*, rats were anesthetized with pentobarbital sodium (45 μ g/g body wt ip) before blood sampling and excision of the tibialis anterior muscle, soleus muscle, and heart. The tissues were weighed and quickly frozen in liquid nitrogen.

P and E_2 Assays

Plasma P was assessed using a human kit from Chiron Diagnostics (East Walpole, MA) in the Hormones Core Laboratory of the Centre Hospitalier Universitaire de Clermont-Ferrand. Plasma E_2 was measured in the RIA unit of Cerba Laboratory (Cergy-Pontoise, France).

GS Assay

GS activity was measured by the formation of [14 C]glutamine from [14 C]glutamate (27). Skeletal muscle and heart were homogenized as previously reported (20). Specific activity of GS was expressed as nanomoles of glutamine formed per hour per milligram of protein. Protein was measured by the bicinchoninic acid method (Pierce, Rockford, IL) according to the manufacturer's instructions.

Northern Blot Analysis and Quantitation of GS mRNA.

Total RNA was extracted as previously described (20). Twenty micrograms of total RNA were electrophoresed in 1% agarose gels containing formaldehyde. RNA was electrophoretically transferred to a nylon membrane (GeneScreen, NEN Research Products, Boston, MA) and covalently bound to the membrane after ultraviolet cross-linking. The membranes were hybridized with a cDNA probe encoding GS (2). The hybridizations were performed at 65°C with [32 P]cDNA fragments labeled by random priming (Ready To Go, Pharmacia, Piscataway, NJ). After washings at the same temperature, the filters were autoradiographed for 3–48 h at –80°C with intensifying screens on Hyperfilm b-max films (Amersham, Buckinghamshire, UK). After stripping of the probe, filters were reprobed with a mouse rRNA 18S probe. Autoradiographic signals were quantified in arbitrary units by use of digital image processing and analysis (NIH Image 1.43) and were normalized using the corresponding rRNA 18S signals to correct for slight variations in RNA loading.

Table 1. Body weight, food consumption, and plasma progesterone and estradiol concentrations in sham-operated, adrenalectomized, or/and ovariectomized rats

	Adult				Aged				Variance Analysis Significant Effects
	Sham	ADX	OV	ADXOV	Sham	ADX	OV	ADXOV	
Body weight, g									
Day 0	353 ± 26	344 ± 33	350 ± 23	348 ± 32	395 ± 58	402 ± 42	387 ± 28	396 ± 48	A
Day 7	353 ± 23	336 ± 36	360 ± 20	335 ± 28	373 ± 30	380 ± 22	376 ± 28	369 ± 42	A
Food consumption, g/rat									
Day -1		19 ± 4				18 ± 1			NS
Day 2	17 ± 4	14 ± 4	16 ± 4	11 ± 5	12 ± 5	5 ± 5	12 ± 3	3 ± 2	A, S
Day 7	20 ± 2	17 ± 5	21 ± 4	17 ± 3	17 ± 4	14 ± 3	21 ± 2	12 ± 5	A, S
Progesterone, nmol/l	148 ± 112	72 ± 47	47 ± 24	1.3 ± 0.3	118 ± 13	236 ± 93	71 ± 39	1.1 ± 0.8	A, S, A × S
Estradiol, pmol/l	130 ± 45	<20	<20	<20	77 ± 6	<20	<20	<20	NS

Values are means ± SE ($n = 6-9$). Female adult and aged rats were either sham-operated (Sham), adrenalectomized (ADX), ovariectomized (OV), or adrenalectomized and ovariectomized (ADXOV) under anesthesia and studied 7 days after surgery. Body weight was measured just before surgery on (day 0) and 7 days after surgery (day 7). Day -1, the day before surgery; day 2, the 2nd day after surgery. Food consumption was measured on days -1, 2, and 7. Progesterone and estradiol were measured in plasma at the end of experiment as described in MATERIALS AND METHODS. ANOVA was performed to discriminate among effects of aging (A), surgery (S), and their interaction (A × S). The significant effects at $P < 0.05$ were indicated by the letters A, S, or A × S; NS, not significant.

Statistical Analysis

Values are given as means ± SE. Factorial ANOVA was performed to assess the effects of aging and surgery, aging and hormone replacement, and their interactions. Comparisons between the two means were carried out using Student's *t*-test (one-tail unpaired test). The level of significance used was 0.05.

RESULTS

Characteristics of Animals

Food intake was similar in adult and aged rats at the beginning of the experiment (day -1) (Table 1). By contrast, both adult and aged animals consumed less food 2 days after surgery. Then, this consumption increased again, even though a slight difference in food intake was still detectable (mostly in aged animals) 7 days after surgery. However, no body weight loss was detectable in adult and aged rats, regardless of the type of surgery, 7 days after surgery (Table 1), although body weight of aged rats at day 0 was slightly higher compared with adult rats.

Animals used in the hormone replacement experiment had similar body weight to those in the main experiment [adult Sham: 323 ± 6 and 330 ± 7 g, respectively, at days 0 and 7, nonsignificant (NS); adult ADXOV: 349 ± 6 and 332 ± 6 g, respectively, at days 0 and 7, NS; aged Sham: 387 ± 11 and 376 ± 14 g, respectively, at days 0 and 7, NS; aged ADXOV: 349 ± 6 and 332 ± 6 g, respectively, at days 0 and 7, NS].

The mass of tibialis anterior and soleus muscles was significantly ($P < 0.05$) lower in aged than in adult controls (-20 and -11%, respectively, in Sham animals), whereas heart mass was slightly higher (+9%) in aged animals (Table 2). In contrast, the type of surgery had no effect on tibialis anterior and soleus muscles and on heart muscle mass.

Basal P level, as observed in Sham rats, was independent of the age of rats (Table 1). In contrast, this parameter decreased in adult ADX rats, whereas it increased significantly in aged ADX rats. Moreover, OV resulted in a significant decrease in P level in adult and aged rats compared with the Sham rats. This suggests that both ovaries and adrenal glands produced P in adult and aged rats. In ADXOV rats, P level was close to zero, whatever the age of the animals. Like P, the basal E_2 level was similar in adult and aged Sham rats. In contrast, E_2 concentrations were not detectable in ADX, OV, and ADXOV rats, whatever the age of animals.

Effect of ADX and OV in Muscle GS Activity from Adult and Aged Rats

After sham operation, adult and aged rats exhibited similar GS activity in tibialis anterior muscle (Fig. 1A), soleus muscle (Fig. 1B), and heart (Fig. 1C). In tibialis anterior muscle, ADX noticeably decreased GS activity in adult and aged rats (50–60%; $P < 0.05$), whereas OV had no effect on GS activity in either adult or aged rats.

Table 2. Effect of surgery on muscle weight of tibialis anterior, soleus and heart from adult and aged rats

Muscle Mass	Adult				Aged				Significant Effects
	Sham	ADX	OV	ADXOV	Sham	ADX	OV	ADXOV	
Tibialis anterior	653 ± 44	664 ± 70	700 ± 40	620 ± 57	525 ± 162	590 ± 33	582 ± 31	588 ± 37	A
Soleus	142 ± 12	153 ± 19	148 ± 10	146 ± 20	126 ± 12	137 ± 22	131 ± 11	130 ± 20	A
Heart	886 ± 92	947 ± 88	930 ± 10	843 ± 115	965 ± 167	972 ± 51	996 ± 95	984 ± 111	A

Values are means ± SE ($n = 6-9$) expressed in mg. ANOVA was performed to discriminate among effects of aging (A), surgery (S), and their interaction (A × S). Significant effects at $P < 0.05$ were indicated by the letters A, S or A × S; NS, not significant.

Fig. 1. Effects of surgery on glutamine synthetase (GS) activity in the tibialis anterior (A), soleus (B), and heart (C) muscles from female adult and aged rats. Animals were either adrenalectomized (ADX), ovariectomized (OV), or adrenalectomized and ovariectomized (ADXOV). Control rats were sham-operated (Sham). Values are means $\pm$ SE (vertical bars) for $n = 6-9$ rats in each group. ANOVA was performed to discriminate among effects of aging (A), surgery (S), and their interaction (A $\times$ S). A significant effect of S at $P = 0.05$ was observed in all tissues. Comparison of 2 means was carried out using Student's t -test: * $P < 0.05$ vs. adult Sham; * $P < 0.05$ vs. aged Sham.

In soleus muscle, GS activity was reduced by ADX in both adult and aged rats (30%; $P < 0.05$), whereas OV had no effect, regardless of animal age. In the heart, neither ADX nor OV modified GS activity in adults. In contrast, the GS activity in heart from aged animals declined by $\sim 40\%$ after ADX. Noticeably, ADXOV resulted in the same effects on GS activity as did ADX alone with regard to tibialis anterior and soleus muscles, regardless of animal age.

mRNA of GS in Tibialis Anterior Muscle from Adult and Aged Rats.

To determine whether GS gene expression correlates with GS activity in the basal state, as previously reported in stress conditions (20), we performed Northern blots to measure the mRNA of GS in the tibialis anterior muscle from adult and aged rats (Fig. 2, A and B). In adult rats, we observed, as previously demonstrated through GS activity, that only ADX was able to decrease mRNA levels of GS (Fig. 2A, top) to the same extent as GS activity. In aged animals, the same result was obtained (Fig. 2B, bottom).

Effect of Hormone Replacement on GS Activity in Adult and Aged Rats

To ascertain whether P or E_2 is involved in the GS response in skeletal muscle, we supplemented adult ADXOV rats (producing neither glucocorticoid nor sex steroids) with P or E_2 . For aged rats, only experiments with E_2 were performed, because no correlation between P levels and GS activity was found (even in the presence of important changes of P in relation to the type of surgery, as reported in Table 1). To evaluate whether hormone replacement induced recovery of GS response in skeletal muscle, values were always compared with those of sham-operated rats (Fig. 3, A and B). Neither P nor E_2 restored GS activity in adults, suggesting that sex steroids do not play a significant role in vivo in GS regulation in adults. With regard to E_2 , this was confirmed in aged rats, because vehicle or E_2 gave rise to similar responses.

To determine whether glucocorticoid replacement was sufficient to restore the sham response of GS in skeletal muscle from ADXOV rats, we assessed the effect of a mild DEX supplementation (0.1 mg/kg) on this enzyme activity in tibialis anterior and soleus muscles. Because the response of GS to glucocorticoids in excess (20, 21) or deficiency (our current results) was similar in young and old rats, the latter experiment was carried out only in adults. We previously verified that there was no difference in GS activity between a control group without surgery and the sham-operated group [248 ± 26 vs. 239 ± 91 (NS) and 125 ± 20 vs. 145 ± 18 (NS) nmol $\cdot$ h $^{-1}$ $\cdot$ mg protein $^{-1}$ in tibialis anterior and soleus muscles, respectively]. We demonstrated a total recovery of GS activity in both muscles (Table 3), which represents an increase higher than 100% compared with vehicle ADXOV rats.

DISCUSSION

To our knowledge, the present study demonstrates for the first time that the hormonal changes in sex steroids (progesterone, estradiol) associated with aging do not affect GS activity in either skeletal muscle or the heart on the basis of in vivo rat studies. To investigate the biological role of these sex steroids in vivo, we tried to suppress surgically the endogenous production of each hormone or both in combination. Such an approach is not easy, because two different organs (adren-

Fig. 2. Effects of surgery on mRNA levels for GS in the tibialis anterior muscle from adult (A) and aged rats (B). Top: Northern blots: Sham rat (lane 1), ADX rat (lane 2), OV rat (lane 3), ADXOV rat (lane 4); they consist of representative experiments at each age. Bottom: quantification of mRNA levels for GS. Autoradiographic signals for the 2.4-kb transcript were quantified in arbitrary units after correction for 18S rRNA abundance for each experiment. Values are means $\pm$ SE (vertical bars) for $n = 4-5$ rats in each group. For each age, comparisons between means were carried out using 1-way ANOVA: * $P = 0.05$ vs. Sham.

nals and ovaries) can produce progesterone, whereas estradiol is produced only by ovaries (15). Consequently, adrenalectomy or ovariectomy could induce only a partial, rather than a complete, progesterone depletion. Our findings provide direct evidence for this concept.

In addition, the alterations in the hypothalamic-pituitary-gonadal function that occur with aging in female rats result in cessation of regular estrous cyclicity (14, 31). The ovaries of aging rats, however, are believed to retain their functional capacity to develop follicles and corpora lutea and to secrete steroid hormones (15). Our current data obtained in sham-operated rats provide support for this concept. Indeed, although progesterone level was similar in adult and aged rats, estradiol was slightly, but significantly, lower in aged than in adult rats. Concerning the effect of aging on progesterone level, this confirms our previous report (20).

The difference in progesterone between adult and aged ADX female rats may seem surprising. Progesterone level was indeed very high in aged ADX rats, as if

these animals spontaneously had become pseudopregnant. This state, well known in aged intact female rats, is characterized by high levels of progesterone and low levels of estradiol, as reported by Lu et al. (15). Another explanation for the higher progesterone levels could be a state of overstimulated activity of the hypothalamic-pituitary-gonadal axis induced by adrenalectomy. Indeed, the age-related changes in estradiol and, to a lesser extent, in progesterone production may activate gonadotropins in the basal state, and this state of basal activation may be further enhanced after adrenalectomy. Thus the ovarian production of progesterone in aged ADX rats may be overstimulated compared with that in young ADX female rats. Such a difference in progesterone level, associated with a similar decrease of GS activity, allows us to conclude that progesterone does not modify GS response in muscle in vivo. However, it must be emphasized that discrepancies in the response of muscle GS to progesterone exist between the current in vivo data and some in vitro experiments, because this hormone plays the role of a mediator in the regulation of GS in cultured muscle cells in vitro

Fig. 3. Effects of 17β -estradiol (E_2 ; $10 \mu\text{g}\cdot\text{kg body wt}^{-1}\cdot 48 \text{ h}^{-1}$ on days 2, 4, and 6 after surgery) or progesterone (P; $140 \mu\text{g}\cdot\text{kg body wt}^{-1}\cdot 48 \text{ h}^{-1}$ on days 2, 4, and 6 after surgery) supplementation on GS activity in the tibialis anterior (A) and soleus (B) muscles from ADXOV rats. Control rats were either sham operated or adrenalectomized-ovariectomized and received vehicle. Values are means $\pm$ SE (vertical bars) for $n = 4-6$ rats in each group. ANOVA was performed to discriminate among effects of aging (A), treatment (T), and their interaction (A $\times$ T). A significant effect of T at $P < 0.05$ was observed in tibialis anterior and soleus muscles. Comparison of 2 means was carried out using Student's t -test: * $P < 0.05$ vs. adult Sham; ** $P < 0.05$ vs. aged Sham.

(10, 19). So, with regard to progesterone and GS activity, our current report demonstrates that muscle cells in culture do not reflect events occurring in the intact organism. However, this may also be related to differences in progesterone levels. Indeed, in earlier studies (10, 19), fibroblasts were incubated in the presence of 10^{-5} M progesterone to observe a partial agonistic effect on GS; this concentration is very high, ~ 75 -fold, compared with physiological levels even in sham rats. Concerning estradiol, a decline in its plasma level has no effect on GS activity regardless of the age of animals. By contrast, the insensitivity of GS to estradiol was similar in muscle in vivo and in cells in culture

(19), regardless of the hormone concentration. In summary, this current work is the first in vivo evidence that neither progesterone nor estradiol acts on skeletal muscle GS in vivo.

Recently, glucocorticoids in excess, which are known to induce GS activity and expression in young and adult rats (1, 5, 18), have been shown to induce a similar effect in skeletal muscle from aged rats (20, 21). In the present experiment, we demonstrate that the absence of glucocorticoids (ADX and ADXOV rats) decreases GS activity to the same extent in skeletal muscle in adult and aged rats. The administration of DEX (0.1 mg/kg) to the ADXOV rats allowed a complete recovery of GS sham response in adult rats. Previous studies reported that the response of GS to such a dose approximated that of GS to endogenous glucocorticoids in skeletal muscle (17). Taken together, these results support the major role of glucocorticoids as a mediator of GS response from adult and aged rats. Moreover, it should be pointed out that, even under standard conditions, endogenous glucocorticoids at physiological levels played a significant role, albeit not an exclusive one, in the GS response of skeletal muscle from female rats.

Contrary to skeletal muscle, heart GS activity was unaltered in response to adrenalectomy in adults, whereas an adrenal-dependent sensitivity of GS appeared in ADX and ADXOV aged animals (the enzyme response declined by 40% in both cases). Because 1) GS activity is not dependent on an excess of glucocorticoids in heart from female aged rats and 2) RU-38486, an antagonist of the glucocorticoid receptor, failed to decrease GS activity in heart from adult and aged rats (20), we conclude that GS activity in aged heart is not regulated by glucocorticoids. Progesterone could not account for this result either, because its plasma level is either very high or near zero in aged ADX and ADXOV rats, respectively. Consequently, this adrenal-dependent response of the aged heart must be due to factors other than glucocorticoids or progesterone. Indeed, the adrenal cortex also synthesizes and secretes mineralocorticoid hormones (aldosterone). Moreover, it was shown that myocardium sensitivity to aldosterone increased with aging because of an increased affinity of mineralocorticoid receptors to aldosterone in old age (6, 25). Consequently, adrenalectomy in aged female rats may evidence the strengthened mineralocorticoid re-

Table 3. Effect of DEX supplementation on GS activity in skeletal muscle from ADXOV adult rats

Treatment	Glutamine Synthetase Activity, $\text{nmol}\cdot\text{h}^{-1}\cdot\text{mg protein}^{-1}$	
	Tibialis anterior	Soleus
Sham	239 ± 91	145 ± 18
Vehicle	$99 \pm 28^*$	$61 \pm 10^*$
DEX	208 ± 55	166 ± 30

Rats were either sham-operated and injected with vehicle, or ADXOV and injected with vehicle or dexamethasone (DEX) by a single injection of $0.1 \text{ mg/kg body wt}$ on day 6. Glutamine synthetase (GS) activity was determined as described in MATERIALS AND METHODS. Values are means $\pm$ SE ($n = 4-6$). * $P < 0.05$ vs. Sham.

sponsiveness of heart by the intermediary of GS activity.

In conclusion, the reproductive endocrine status of female rats does not affect muscle glutamine synthesis during aging. In contrast, the major role of glucocorticoids in GS regulation in skeletal muscle (with mixed or oxidative fibers) is evidenced in adult and aged rats in basal conditions. According to the glucocorticoid hypothesis of hippocampal aging (33), we can rule out the possibility that alterations in glucocorticoid secretion contribute to modify basal GS responsiveness in aged muscle. It is tempting to speculate that, during aging, a high degree of muscle GS activity is preserved to maintain a sufficient supply of glutamine to accommodate the demand of other tissues, despite progressive sarcopenia, and regardless of the representative status.

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