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EFFECTS OF PHYSIOLOGICAL AND PATHOLOGICAL LEVELS OF GLUCOCORTICOIDS ON SKELETAL MUSCLE GLUTAMINE METABOLISM IN THE RAT

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Abstract—The effects of physiological and pathological concentrations of glucocorticoids were investigated using the glucocorticoid antagonist RU486 and the synthetic glucocorticoid dexamethasone, respectively. The effects of these treatments on the concentrations of glutamine and other amino acids in skeletal muscle and plasma and on the rates of release of glutamine and alanine from incubated preparations of skeletal muscle of the rat were investigated. Dexamethasone treatment increased the concentration of glutamine and the rate of release of this amino acid from incubated soleus muscle preparations. This treatment decreased the concentration of glutamine in both gastrocnemius and EDL muscles, but was without effect on the rate of glutamine release from EDL muscles. In contrast, administration of the glucocorticoid antagonist RU486 decreased the rate of glutamine release from muscle. It is concluded that glucocorticoids have marked effects on the metabolism of glutamine by skeletal muscle *per se* and that these hormones may be important in the control of the rate of glutamine release from muscle in both physiological and pathological conditions.

Glutamine is utilized by a large number of different tissues, including the intestine [1, 2], kidney [3, 4], liver [5] and cells of the immune system [6–8]. Furthermore, mitogen-stimulated proliferation of lymphocytes in culture is dependent upon the presence of glutamine and the rate of proliferation is dependent upon the concentration of glutamine in the culture medium [9]. Cells of the immune system may therefore be a major site of glutamine utilization *in vivo* and consequently maintenance of the normal plasma level of this amino acid may be necessary to permit a rapid and efficient response to an immune challenge. Glutamine may also be used at high rates by endothelial cells [10] and fibroblasts [11, 12], so that the rate of utilization of plasma glutamine would be expected to increase not only during conditions such as sepsis, but also following other types of injury in order to provide for the processes of wound healing and tissue repair.

Much of the glutamine that enters the body via dietary sources is utilized by cells of the small intestine, so that little of this normal source of amino acids is available to maintain the blood glutamine level [13]. There is now considerable evidence that skeletal muscle provides much of the glutamine required by other tissues: skeletal muscle synthesizes and releases glutamine at a high rate [14–16]. These considerations lead to the suggestion that following injury the rate of release of glutamine by muscle should increase: there is some evidence to support this point of view [17–19]. However, the agent(s) responsible for the increased rate of glutamine release are not known.

Glucocorticoids are considered to be important in the metabolic response to injury [20, 21] and measurements of arteriovenous differences of glutamine across the limbs of experimental animals and man suggest that glucocorticoids increase the net rate of glutamine release [22–25]. It is presumed that this increase in rate of release is due to effects on skeletal muscle: but since there is evidence that adipose tissue and skin can use glutamine at high rates [26, 27], changes in rates of utilization by these tissues could contribute to changes in the arteriovenous differences. The only report of an effect of the administration of glucocorticoids to rats on the rate of glutamine release from isolated, incubated muscle showed, in contrast to the above reports, that cortisone decreased the rate of glutamine release [28]. However, this latter study, in common with many others, employed pathological levels of glucocorticoids.

Consequently, it was decided to study systematically the effects of both physiological and pathological levels of glucocorticoids *in vivo* on the rate of glutamine release from incubated skeletal muscle isolated from these animals. The concentrations of glutamine and other amino acids in muscle and plasma have also been measured.

MATERIALS AND METHODS

Materials. All chemicals, biochemicals and enzymes were obtained from sources previously given [19]. In addition, dexamethasone acetate was obtained from the Sigma Chemical Co. (Poole, U.K.) and RU486 was kindly donated by Dr R. Deraedt, Roussal Uclaf (Rommaneville, France).

Animals and treatment procedures. Male Wistar rats (70–80 and 160–180 g) were obtained from Harlan-Olac (Bicester, U.K.).

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Glucocorticoid excess was induced in rats by daily, intraperitoneal injections of dexamethasone acetate (2.5 mg/kg body wt) for 5 consecutive days. This dose is approximately equivalent to endogenous glucocorticoid levels in animals under severe stress [29], but since dexamethasone is a synthetic glucocorticoid analogue the effects of this treatment may be described as pharmacological. Rats were fasted for 12–14 hr prior to experimentation, which was performed 24 hr after the final injection. Treated rats lost approximately 20 g during the treatment period, whilst control rats gained approximately 8 g. However, dexamethasone was without effect on food intake.

The glucocorticoid receptor antagonist RU486 [30] was employed to study the effects of physiological levels of glucocorticoids. Rats used in these experiments were kept under a "reversed" light–dark cycle (lights on 8.00 p.m. to 8.00 a.m.). Rats were given a single intraperitoneal injection of RU486 (50 mg/kg body wt) at 6.00 a.m.; which is 2 hr prior to the normal diurnal peak in the glucocorticoid level. Rats were killed and muscle removed for incubation at 2.00–3.00 p.m.; this is approximately 6 hr after the peak in plasma levels of glucocorticoids, which is considered to be the normal time lag in the physiological response to these hormones (see Ref. 31). Control and treated animals were given food and water *ad lib.* prior to the experiment. Food intake was recorded and was not different between groups.

Incubation of muscles. Isolated, incubated muscle preparations were employed in the present study. This technique has a number of advantages; for example, the metabolism of skeletal muscle can be studied in isolation from other tissues and the response of different muscle types can be studied separately. The muscle preparation also allows the determination of the absolute rate of glutamine release, in contrast to the net rate of release determined from arteriovenous difference studies. Importantly, the concentration of glutamine in muscle was maintained during the incubation procedure [pre-incubation 4536(412), post-incubation 4814(174) nmol/g].

Stripped soleus muscle preparations were obtained from 160–180 g rats [32] and whole soleus muscle and extensor digitorum longus (EDL) muscle preparations were obtained from 70–80 g rats [33]. All muscles were incubated in Krebs–Henseleit bicarbonate buffer, containing glucose (5.5 mM), defatted bovine serum albumin (1.5%) and insulin (10 μUnits/mL) for 60 min.

Measurement of the concentration of metabolites. The rates of release of amino acids from incubated muscle strips were determined by the enzymatic analysis of the concentrations of glutamine [1] and alanine [34] in the incubation medium.

Gastrocnemius muscles were freeze-clamped at liquid nitrogen temperatures and blood samples were taken from the heart immediately after the animals were killed. Muscle and plasma samples were prepared and extracted as described previously [19], and subsequently analysed for concentrations of glutamine [1], alanine [34] and branched chain amino acids [35].

RESULTS

Dexamethasone treatment in older rats (180 g), decreased the concentration of glutamine in gastrocnemius muscle, but the plasma concentration was unaffected. In contrast, alanine concentrations in plasma and muscle were increased following dexamethasone treatment (Table 1). This treatment increased the rate of release of glutamine but decreased that of alanine from the incubated soleus muscle (Table 2).

Dexamethasone treatment in younger rats (90 g) also increased the rate of glutamine release from the incubated soleus muscle but, in contrast, had no effect on the rate of release from incubated EDL muscle. The glutamine concentration in EDL muscle measured at the end of the incubation was decreased by dexamethasone treatment of young rats [control 3194(351), dexamethasone treated 2143(86) nmol/g, $P < 0.01$]: this effect is similar to that observed for gastrocnemius muscle in older rats (Table 1). In contrast, the concentration of glutamine in soleus muscle at the end of the incubation was increased by dexamethasone treatment [control 3517(207), dexamethasone treated 4420(392) nmol/g, $P < 0.05$]. A similar increase was also observed in soleus muscle removed from the animal and immediately freeze-clamped, but it was not statistically significant [control 3002(323), dexamethasone treated 3896(483) nmol/g, $P > 0.05$].

Administration of the glucocorticoid receptor antagonist, RU486, to rats decreased the concentration of glutamine in both muscle and plasma; it also decreased the rate of glutamine release from the incubated soleus muscle (Tables 1 and 2). The concentrations of branched chain amino acids in both muscle and plasma and the plasma concentration of alanine were also decreased by RU486, whereas the concentration of alanine in muscle was not affected.

DISCUSSION

Administration of dexamethasone to rats increased the rate of glutamine release from incubated soleus muscle isolated from these animals but it had no effect on the rate of release from incubated EDL muscle. This is the first report of a glucocorticoid-induced increase in the rate of glutamine release from incubated skeletal muscle and demonstrates that glucocorticoids may influence red and white muscle fibres differently, at least in relation to glutamine metabolism. This may be due to a greater number of glucocorticoid receptors in red muscle [36]. The increased rate of glutamine release from isolated soleus muscle following glucocorticoid treatment is consistent with results of experiments using either perfused rat hindquarter [24] or arteriovenous concentration differences [22–25]; the present results provide evidence that the increase is due to an effect on skeletal muscle *per se*.

The different effect of glucocorticoids on red and white muscle fibres is also seen in changes in the glutamine concentration: in the soleus muscle (containing mainly red fibres) the glutamine concentration was increased but it was decreased in

Table 1. The effects of dexamethasone and the glucocorticoid antagonist RU486 on the concentrations of glutamine, alanine and branched chain amino acids in gastrocnemius muscle and plasma of 180 g rats

	Amino acid concentration (nmol/g or nmol/mL)					
	Glutamine		Alanine		BCAA	
Treatment	Muscle	Plasma	Muscle	Plasma	Muscle	Plasma
Control	3807 ± 152 N = 11	885 ± 46 N = 13	1447 ± 96 N = 11	308 ± 21 N = 13	465 ± 22 N = 11	452 ± 22 N = 11
Dexamethasone	2909 ± 109‡ N = 16	903 ± 27 N = 18	1830 ± 61† N = 16	396 ± 20† N = 20	447 ± 14 N = 16	392 ± 10* N = 21
Control	5373 ± 274 N = 20	1058 ± 41 N = 19	1989 ± 120 N = 18	522 ± 30 N = 10	317 ± 25 N = 5	371 ± 6 N = 5
RU486 treated	4381 ± 254† N = 24	901 ± 42† N = 24	1800 ± 85 N = 20	431 ± 37* N = 10	245 ± 23* N = 5	265 ± 8‡ N = 5

Values are means ± SE. The significance of the differences between means is denoted by *($P < 0.05$), † ($P < 0.01$) or ‡ ($P < 0.001$).

Table 2. The effects of dexamethasone and the glucocorticoid antagonist RU486 on the rates of glutamine and alanine release from incubated soleus and extensor digitorum longus (EDL) muscles

		Rate of amino acid release (nmol/min/g)			
		Soleus		EDL	
Treatment	Weight of rats (g)	Glutamine	Alanine	Glutamine	Alanine
Control	180	33.2 ± 1.4 N = 13	25.1 ± 1.7 N = 14	ND	ND
Dexamethasone	180	39.2 ± 1.3‡ N = 24	20.0 ± 1.0† N = 24	ND	ND
Control	80	62.2 ± 3.2 N = 24	ND	21.9 ± 1.9 N = 27	ND
Dexamethasone	80	78.9 ± 4.2‡ N = 12	ND	23.3 ± 1.7 N = 27	ND
Control	180	41.8 ± 1.6 N = 27	ND	ND	ND
RU486 treated	180	36.8 ± 1.7* N = 26	ND	ND	ND

Values are mean ± SE. The significance of the differences between means is denoted by * ($P < 0.025$), † ($P < 0.02$) or ‡ ($P < 0.01$).
ND, not determined.

gastrocnemius and EDL muscles (containing mainly white fibres).

The effects of the glucocorticoid receptor antagonist, RU486, are considered to be caused by a decrease in the effect of physiological levels of plasma glucocorticoids (in many other studies it has been common practice to employ pathological or suprapathological levels of these hormones). Administration of RU486 decreased the concentrations of glutamine in both muscle and plasma and decreased the rate of glutamine release from the incubated soleus muscle. This latter finding suggests that the physiological diurnal increase in plasma glucocorticoid levels results in an increased rate of glutamine release from soleus muscle. Thus, it is suggested that glucocorticoids, either at physiological or pathological concentrations, cause an increase in the rate of glutamine release from soleus muscle.

Administration of low doses of glucocorticoids to patients suffering from infectious mononucleosis accelerated their recovery [37]. In addition, the survival of adrenalectomized monkeys post-surgery was increased following administration of sufficient glucocorticoids to raise the plasma level within physiological limits [38]. It is suggested, therefore, that these beneficial effects of physiological levels of glucocorticoids are caused by an increased rate of glutamine release from muscle which, in turn, provides a higher plasma concentration of this amino acid to permit rapid rates of proliferation of cells of the immune system and those involved in tissue repair. It is possible, therefore, that this is one important role of the raised levels of glucocorticoids seen in injury, sepsis and post-surgery.

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