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European Journal of Pharmacology 366 (1999) 19–26

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Effects of controllable stress on masticatory behaviour and muscle structure: partial protective effect of clomipramine

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Received 9 July 1998; revised 23 November 1998; accepted 27 November 1998

Abstract

The influence of controllable painless stress and clomipramine treatment was evaluated on masticatory behaviour and myosin heavy chain expression in masticatory rat muscles: anterior digastric, anterior temporalis and masseter superficialis. The adult fast isoforms of myosin heavy chains detected were myosin heavy chains 2A, 2X and 2B. The myosin heavy chains composition of anterior temporalis muscle was unchanged by stress or by treatment. In anterior digastric and masseter superficialis muscles, stress induced an increase in 2B and a decrease in 2X and 2A. Under stress, whereas the myosin heavy chains composition of anterior temporalis and anterior digastric muscles was unaffected by clomipramine, this drug modified significantly the myosin heavy chains composition of masseter superficialis muscle which became comparable to that of control muscle. Stress-induced myosin heavy chains transformations led to an increased velocity of anterior digastric and masseter superficialis muscles but not anterior temporalis muscle. Gnawing and mastication were increased by stress and incisor grinding was reduced. Stress shortened the duration of gnawing and increased the fatigability of anterior digastric and masseter superficialis muscles, whereas clomipramine increased the duration of mastication and reduced the fatigability of masseter superficialis muscle. Stress produces selective changes in masticatory muscles and behaviour. This study demonstrates the muscle type-specific protective effect of clomipramine against stress-induced structural transformations of masseter superficialis muscle and the specific concomitant behavioural modifications. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Clomipramine; Myosin heavy chain; Masticatory behaviour; (Rat)

1. Introduction

Evidence is now available showing that muscle contractile properties and myosin heavy chain composition are correlated (Bottinelli et al., 1996). The four major myosin heavy chains isoforms detectable in adult skeletal muscles are three fast types, myosin heavy chains 2A, 2X and 2B, and one slow type, myosin heavy chain 1 (Bär and Pette, 1988; Schiaffino et al., 1989). Despite their similarity in primary structure, the expression of the different myosin heavy chains isoforms is precisely regulated in a tissue- and developmental stage-specific manner (Whalen et al., 1981). In addition, various factors, such as altered physiological stimuli, various hormones (Izumo et al., 1986) and altered loading states (Baldwin, 1996), are known to cause changes in myosin heavy chains isoforms. For instance, when the working conditions are changed, marked transitions in the myosin content occur in rat fast- and slow-twitch muscles (Swynghedauw, 1986). These modifications generally adapt the muscle to the new environmental requirements. Thus, after a few weeks of synergistic tenotomy, fast muscles become slow, fatigue resistant, and thus better adapted to endurance. Sfondrini et al. (1996) proposed that rat skeletal muscles could quickly adapt to functional demands by changing the composition of the fast fibres. Adult skeletal muscle expresses three fast myosin heavy chains isoforms, providing considerable structural and functional diversity. Indeed, myosin heavy chain 2 is expressed in muscle regions used during sustained locomotion (2A and 2X) or high-power-output activity (2X and 2B) (Adams et al., 1994) whereas myosin heavy chain 2X is predominantly expressed during stimulation-induced fast-to-slow and slow-to-fast fibre transformation (Schiaffino et al., 1989).

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PII: S0014-2999(98)00890-5

Stressful situations result from particular changes in the environmental requirements. Stress has been related to depression in humans and depression-like behaviours in animals (Anisman and Zacharko, 1982). Exposure to stress induces changes in different physiological variables, such as plasma hormone levels (Tamasy et al., 1986; Kant et al., 1992; Gomez et al., 1996; Josko, 1996). Feinmann et al. (1984) have shown, in humans, that muscular disorders, especially facial pain syndromes, are associated with psychological stress. One may then wonder if stress is accompanied by a modification of muscle structure.

It has been shown that the activity of masticatory muscles is affected by stress. Indeed, Ruf et al. (1997) have reported a correlation between emotional stress and electromyographic activity of the masticatory muscles in human volunteers: the electromyographic activity during the stress situation was significantly greater than during the non-stress situation. Furthermore, restraint stress (150 min daily) has been shown to decrease food and water intake (Michajlovskij et al., 1988). This decrease was found even after a single immobilisation period and lasted up to the 7th day. Structural changes in masticatory muscles could therefore be a sequel of these modifications of activity.

Feinmann et al. (1984) have reported that facial muscle disorders can be successfully relieved by treatment with a tricyclic antidepressant. This pain relief appeared to be independent of any antidepressant effect. Accordingly, the clomipramine treatment procedure used in this study consisted of antinociceptive and non-antidepressant elements. In animals, the antidepressant effects of clomipramine appear after at least 10 days of treatment, and the half-life of myosin heavy chains was calculated by Young and Schneible (1984) to be 30.6 h. The treatment duration was therefore fixed at 7 days to allow observation of the effects of clomipramine on stressed animals. Guiol et al. (1984) reported that clomipramine showed a clear dose-dependent analgesic effect at doses between 10 and 40 mg/kg/day administered subchronically for 5 days. A subchronic twice-daily treatment was therefore chosen with a dose of 20 mg/kg/day. Furthermore, chronic administration of clomipramine prevents some stress-induced physiological modifications, particularly changes in plasma hormone levels (Adell et al., 1989; Lopez and Vazquez, 1990). There is very little information available concerning the effects of antidepressants on masticatory behaviour. It has been shown that antidepressant treatment inhibits food intake (Blavet and Defeudis, 1982). The effects on muscle structure and masticatory behaviour of clomipramine administered during a stressful situation are however unknown.

Therefore, the aim of the present investigation was first to evaluate the effects of chronic stress on the myosin heavy chains composition of masticatory muscles and on behaviours involving these muscles, and secondly to study the influence of concomitant antidepressive treatment. The stressful procedures used in the literature generally involve painful stimuli (electric footshock and tailshock) in which the effects of stress cannot be distinguished from the effects of pain. Therefore, the stress procedure chosen in this study was a painless learning procedure in which the animal could control the stressor (bright light). This procedure causes controllable, painless stress. For the tricyclic antidepressant in this study, we chose clomipramine, which is widely used in the treatment of affective disorders.

2. Materials and methods

2.1. Animal care

Twenty-four 2-month-old female Wistar rats (iops IFFA-CREDO) were used. From birth, the animals were housed under a reversed day–night cycle (dark period 08.00–20.00 h). Food and water were available ad libitum during the whole experiment.

2.2. Experimental procedure

The animals were randomly divided into four groups: two control groups treated with acute saline or clomipramine treatment and no learning ($n = 6$ per treatment), and two experimental groups with bright light active avoidance learning for two weeks treated either with saline or with clomipramine during the second week of learning ($n = 6$ per treatment). Bright light active avoidance learning was carried out in an operant conditioning chamber ($42 \times 27 \times 19$ cm), at the rate of 1 h per day at the beginning of the dark period. Clomipramine or saline was administered 30 min before the start of each learning session. The front wall (27×19 cm) of the chamber was constructed of clear Plexiglas in order to allow the video observation of the animals. The left and right walls of the chamber were equipped with a lever that was centred horizontally, 3 cm above the floor. Aversive illumination was supplied by two 20-W neon tubes placed 10 cm above the chamber. The reward, which was given only after presses on the right lever, consisted of a dark session (light extinction) of 30 s. Presses on the left lever were not reinforced. Only rats that acquired a sufficient level of reinforcement before the start of treatment were used, i.e. at least 70% of the lever presses had to be on the reinforced lever by the end of the first week. These animals were divided into two learning groups. The behavioural sessions were run 30 min after acute treatment for the control groups and 30 min after the last treatment for the learning groups (24 h after the last learning session). Muscle sampling was carried out immediately after behavioural observation.

2.3. Behavioural observation

Lever press performance was recorded each day for each rat. The discrimination performance was calculated

by dividing the number of reinforced lever presses by the total number of lever presses.

The behavioural sessions were run in the operant conditioning chamber and lasted 30 min. Observation and data recording were accomplished by using a video recording system and a 486 PC microcomputer equipped with software programmed in Turbo Pascal. Three different aspects of behaviour, pellet gnawing, mastication and incisor grinding, were recorded in terms of the number of events and the duration of each event. The total number of rearings was also measured in order to evaluate exploratory behaviour, which is indicative of the stress state of the animals.

2.4. Drug administration

Clomipramine (Ciba-Geigy) was dissolved in NaCl 0.9% and injected i.p. in a volume of 0.5 ml/100 g body weight either acutely at the dose of 10 mg/kg for one control group or twice a day at the dose of 20 mg/kg per day for one experimental group. The saline groups (one control and one experimental group) were injected under the same conditions, but without clomipramine.

2.5. Muscle sampling and myosin extraction

After cardiac injection of concentrated KCl, the following masticatory muscles were removed unilaterally (right) from the animals: anterior temporalis (elevator mandibular muscle), anterior digastric (depressor mandibular muscle) and masseter superficialis (propulsive mandibular muscle). After dissection, the muscles were immediately frozen in liquid nitrogen for protein electrophoretic analyses. Myosin was crudely extracted in a high ionic strength buffer, as described by D'Albis et al. (1979).

2.6. Electrophoretic analysis of myosin heavy chains and quantification

Electrophoresis was performed according to the method of Talmadge and Roy (1993). Mini-gels were used in the Bio-Rad Mini-protean II Dual Slab Cell. Electrophoresis took place in a cold room, at a temperature of 6°C for the whole run. To separate all the heavy chains, the duration of the run was 28 h. The gels were stained with Coomassie blue R-250. The relative amounts of the different myosin heavy chains were measured using an integration densitometer Bio-Rad GS-700 and analysed with the Molecular Analyst Program.

2.7. Statistical analysis

The results are expressed as means ± S.E.M.. For biochemical data, after a one-way analysis of variance, Student's *t*-test was used to establish the intergroup comparison. For behavioural data, after the Kruskal–Wallis one-way analysis of variance, the Mann–Whitney *U*-test was used to establish the intergroup comparison. Differences were considered significant at $P < 0.05$.

3. Results

The electrophoretic data are illustrated in Fig. 1 and the densitometric analysis is presented in Table 1. The order of increasing electrophoretic mobility of adult myosin heavy chains isoforms is fast 2A, 2X and 2B types (Fig. 1). The proportions of myosin heavy chains isoforms are expressed as percentages relative to the total amount of myosin heavy chains present in the muscles studied (Table 1).

In the three muscles, three fast myosin heavy chains isoforms could be observed, myosin heavy chains 2A, 2X and 2B (Fig. 1). Myosin heavy chain 2B was the most abundant isoform (> 60%) in the anterior temporalis whereas in the anterior digastric and masseter superficialis, myosin heavy chain 2X was the predominant isoform (> 50%) (Table 1). Myosin heavy chain 2A was the least expressed myosin heavy chain in the three muscles studied (< 13%). The anterior temporalis showed the highest proportion of myosin heavy chain 2B and the lowest proportion of myosin heavy chains 2A and 2X, whereas the masseter superficialis showed the lowest proportion of myosin heavy chain 2B and the highest proportion of myosin heavy chain 2X.

The myosin heavy chains protein isoform content of two of the three muscles studied was significantly affected by the stress procedure. The anterior temporalis myosin heavy chains composition was not affected by the stress

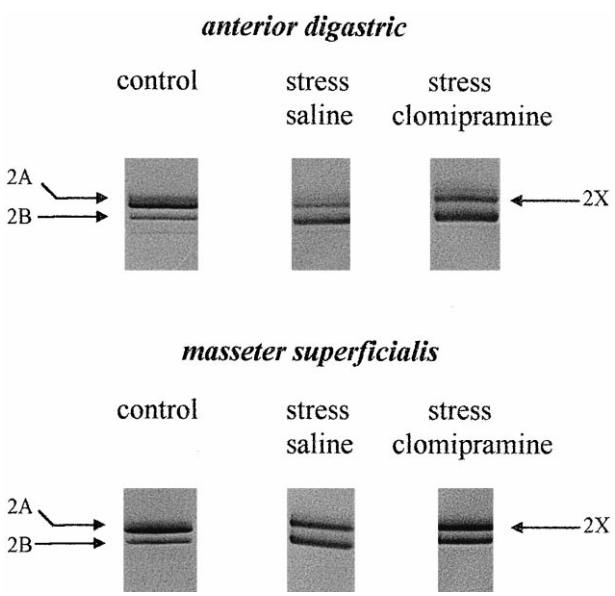

Fig. 1. Effect of stress and clomipramine on myosin heavy chains composition in two rat adult masticatory muscles (anterior digastric and masseter superficialis) in order of increasing electrophoretic mobility: fast 2A, 2X and 2B.

Table 1
Myosin heavy chain distribution in masticatory muscles in saline- and clomipramine-treated rats after controllable stress

Group	Treatment	Myosin heavy chain type (%)		
		2A	2X	2B
Anterior temporalis				
control	saline	8.4 ± 1.1	29.3 ± 1.6	62.3 ± 1.8
control	clomipramine	5.9 ± 1.0	27.9 ± 3.3	66.2 ± 4.0
stress	saline	6.0 ± 1.7	23.0 ± 3.3	71.0 ± 4.6
stress	clomipramine	4.5 ± 0.7	22.0 ± 1.2	73.5 ± 1.6
Anterior digastric				
control	saline	13.0 ± 1.1	52.4 ± 4.6	34.6 ± 4.7
control	clomipramine	14.9 ± 2.5	47.8 ± 4.0	37.3 ± 5.9
stress	saline	5.4 ± 1.2[a]	35.3 ± 4.6[a]	59.3 ± 4.8[a]
stress	clomipramine	7.0 ± 2.3[a]	38.3 ± 2.9	54.7 ± 5.0[a]
Masseter superficialis				
control	saline	8.5 ± 1.1	63.8 ± 3.7	27.7 ± 3.9
control	clomipramine	8.0 ± 1.9	64.4 ± 2.6	27.6 ± 3.9
stress	saline	3.9 ± 0.6	49.0 ± 3.1[a]	47.1 ± 3.4[a]
stress	clomipramine	6.2 ± 0.8	59.3 ± 2.1[b]	34.5 ± 2.7[b]

Values are mean percentages of total myosin heavy chains ± S.E.M. (see Section 2.7, Statistical analysis).
$n = 6$ per group.
[a]Significantly different from control muscles under the same treatment, $P < 0.05$.
[b]Significantly different from saline-treated muscles in the same behavioural situation, $P < 0.05$.

procedure, irrespective of the treatment. In anterior digastric and masseter superficialis, under saline treatment and the stress procedure, the relative proportion of myosin heavy chain 2B increased significantly to the detriment of myosin heavy chains 2A and 2X. However, with clomipramine treatment, the stress procedure led to the same myosin heavy chains content as under saline treatment in anterior temporalis and anterior digastric, except that in anterior digastric the relative decrease in myosin heavy chain 2X was not statistically significant any more. However, in the masseter superficialis muscle of the stressed animals treated with clomipramine, the myosin heavy chains profile was significantly different from that of the stressed saline-treated animals. In this muscle, the myosin heavy chains distribution became comparable to that of the non-stressed animals.

The course of acquisition of the learning task is presented in Fig. 2. The percentage of discrimination increased significantly during the course of the experiment ($F(13) = 6.4$, $P < 0.0001$). During the first week, the percentage of discrimination increased from around 65% to 85% in the two experimental groups. During the second week, discrimination increased from 81% to 95% in the stressed saline-treated group and from 94% to 97% in the stressed clomipramine-treated group. However, no statistically significant difference appeared between these two groups over the 14 days ($F(1) = 0.57$, NS). The total number of lever presses was not statistically significantly different between the two experimental groups ($F(1) = 0.008$, NS).

The stress state of the animals was evaluated by measuring rearing activity. The stressed animals (stress saline: mean ± S.E.M. = 107 ± 23, stress clomipramine: 28 ± 8) showed a significantly reduced total number of rearings with regard to controls (control saline: 141 ± 15, control

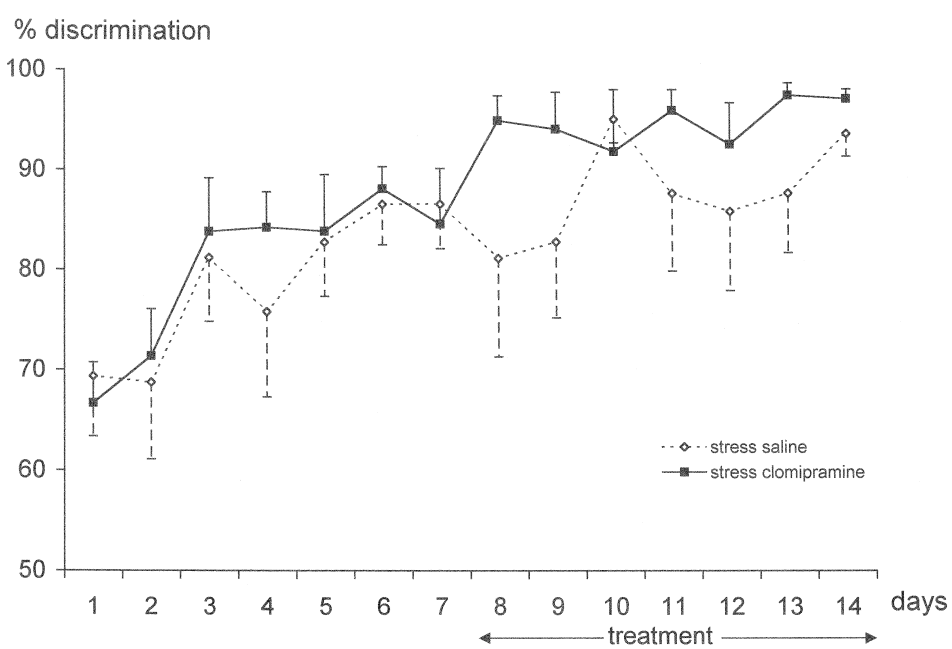

Fig. 2. Evolution of the percentage of discrimination of the reinforced lever in a two-lever operant chamber during a 2-week bright light avoidance learning experiment. Influence of subchronic twice-daily clomipramine treatment (20 mg/kg/day) during the second week. The percentage of discrimination was calculated by dividing the number of reinforced lever presses by the total number of lever presses. Values are means with S.E.M.

Table 2
Effects of clomipramine treatment and controllable stress on three behaviours involving masticatory muscles: gnawing, mastication and incisor grinding

Group	Treatment	Number	Total duration (s)	Median duration (s)
Gnawing				
control	saline	81.5 ± 16.5	175.5 ± 39.3	1.6 ± 0.2
control	clomipramine	58.2 ± 10.3	126.5 ± 22.0	1.9 ± 0.2
stress	saline	140.5 ± 13.3[a]	183.2 ± 23.0	1.0 ± 0.1[a]
stress	clomipramine	102.2 ± 15.7[a]	158.4 ± 30.4	1.2 ± 0.1[a]
Mastication				
control	saline	74.7 ± 15.9	267.3 ± 62.9	2.4 ± 0.7
control	clomipramine	56.7 ± 10.6	245.4 ± 58.1	2.6 ± 0.3
stress	saline	136.0 ± 14.5	446.7 ± 110.2	1.9 ± 0.3
stress	clomipramine	101.7 ± 15.7[a]	485.1 ± 71.4	3.4 ± 0.5[b]
Incisor grinding				
control	saline	2.0 ± 1.7	6.4 ± 3.7	2.1 ± 1.1
control	clomipramine	5.3 ± 0.9[b]	20.4 ± 7.4	2.8 ± 0.7
stress	saline	1.2 ± 0.5	1.3 ± 0.7	0.7 ± 0.3
stress	clomipramine	8.5 ± 5.3	20.5 ± 10.7	1.8 ± 0.9

Values are means ± S.E.M. (see Section 2.7, Statistical analysis).
$n = 6$ per group.
[a]Significantly different from controls under the same treatment, $P < 0.05$.
[b]Significantly different from saline-treated animals in the same behavioural situation, $P < 0.05$.

clomipramine: 90 ± 10) ($H(3) = 13.26$, $P = 0.0041$). Furthermore, clomipramine induced an additional reduction in exploration in the control ($U = 4$, $P = 0.025$) as well as in the stressed animals ($U = 5$, $P = 0.0374$).

The other behavioural data are presented in Table 2. Under saline treatment, the control rats exhibited very few incisor grinding events, as compared to the number of gnawing and mastication events. During the 30 min of observation, the mean total duration of incisor grinding was only 6.4 s, whereas the animals spent 175.5 s on average for gnawing and 267.3 s for mastication. For the three behaviours observed, the median duration of the events was 1.5 to 2.5 s. Under clomipramine treatment, the control rats exhibited significantly more incisor grinding events, whereas the two other behaviours were not significantly affected by this treatment.

In saline-treated rats, the stress procedure induced a marked increase in the number of gnawing events, but these events were significantly shorter. The total duration of gnawing remained stable. Incisor grinding was not significantly modified by the stress procedure in the saline-treated rats. The number of mastication events was increased by the stress procedure, although this increase only reached the level of significance after clomipramine treatment.

In clomipramine-treated rats, the stress procedure induced a similar increase in the number of gnawing events as seen in the saline-treated rats. The median duration of the gnawing events was decreased by the stress procedure whereas the total duration of these events remained stable. The median duration of the mastication events was increased by clomipramine treatment under the stress procedure. The number of events and total duration of incisor grinding were increased by the clomipramine treatment, although these changes did not reach the level of significance.

4. Discussion

With respect to the maximum velocity of muscle fibre shortening, the type 2B fibre is thought to have the highest velocity, followed by 2X > 2A > 1 (Bottinelli et al., 1991). Our results showed that the three muscles studied (anterior temporalis, anterior digastric and masseter superficialis), which exclusively expressed the type 2 myosin heavy chains isoforms, were fast-twitch muscles. Among these fast muscles, the anterior temporalis muscle, which contained mainly type 2B, was faster than the anterior digastric muscle, which contained 35% type 2B and 53% type 2X, which in turn was faster than the masseter superficialis muscle, which contained mainly 2X type.

Our results show that the controllable bright light avoidance procedure is stressful. Exploratory behaviour is reduced by stressful experiences (Van den Berg et al., 1998). It was indeed decreased in the rats that underwent bright light avoidance learning compared to controls. In addition, clomipramine accentuated the effect of stress on exploration, in accordance to the observations of Ferretti et al. (1995). Although it did not reach the level of significance, the learning performance of the stressed animals improved after clomipramine. Indeed, it has already been shown that antidepressants improved learning in rats (for example see Yau et al., 1995).

In saline-treated rats, the stress procedure modified significantly the expression of myosin heavy chains isoforms in two of the three muscles studied. Indeed, whereas the anterior temporalis muscle kept the same myosin heavy

chains profile, the anterior digastric and masseter superficialis muscles became significantly faster than the controls after two weeks of stress. To our knowledge, this is the first time that stress has been shown to be accompanied by specific changes in muscle structure. Sfondrini et al. (1996), on studying the plasticity of masticatory muscles, proposed that rat skeletal muscles could quickly adapt to functional demand by changing their fibre type composition and that the changes appeared restricted to the fast fibre population. They compared the muscles of rats fed on a normal diet to those of rats kept on a liquid diet for 20 days and found that the change from a hard pelleted diet to a liquid diet caused a shift in fibre type and myosin heavy chains distribution, characterised by an increase in myosin heavy chain 2B in anterior temporalis and anterior digastric muscles but not in the masseter superficialis muscle. With regard to these biochemical observations, the changes in masticatory behaviour could be one of the factors influencing the myosin heavy chains composition of masticatory muscles. The stress-induced modification of the expression of myosin heavy chains isoforms observed in our study could therefore be related to changes in masticatory behaviour. Behaviour was then observed in order to verify this hypothesis.

Indeed, the changes in the myosin heavy chains composition of masticatory muscles were accompanied by modifications in masticatory behaviour. Two components of masticatory behaviour, gnawing and mastication, were increased by the stressful situation whereas incisor grinding was rather inhibited. More precisely, gnawing behaviour was shorter lasting but occurred more frequently in stressed animals. Sieck et al. (1996) have classified the muscular motor units physiologically as slow-twitch fatigue resistant (myosin heavy chain 1), fast-twitch fatigue resistant (myosin heavy chain 2A), fast-twitch fatigue-intermediate (myosin heavy chain 2X alone or 2X and 2A), and fast-twitch fatigable (myosin heavy chains 2X and 2B or 2B alone). As the stress procedure increased the relative proportion of myosin heavy chain 2B to the detriment of myosin heavy chains 2X and 2A in anterior digastric and masseter superficialis muscles, this transition was accompanied by changes in muscle resistance to fatigue. This differential fatigability could explain this shortening of the gnawing events. It should be noted that the masseter superficialis muscle, which is insensitive to dietary change (Sfondrini et al., 1996), was markedly affected by the stress procedure and became significantly faster than the control muscle. This result suggests that either masticatory behaviour is not the only factor affecting the structure of the masticatory muscles, or stress and dietary change do not modify masticatory behaviour in the same way. However, from our results it is not possible to determine which hypothesis is correct, i.e. either the behavioural modifications are at the origin of the myosin heavy chains transitions or these transitions induce the behavioural changes observed.

Stress not only modifies masticatory behaviour, it can also induce physiological modifications. Indeed, controllable or uncontrollable stress has been shown to change the activity of some endocrine glands, in particular the thyroid and adrenal glands. The effects of stress on hormone levels depend in particular on its duration (Tamasy et al., 1986; Kant et al., 1992). It has also been suggested that control over stressors attenuates the effects of stress on physiology (Kant et al., 1992; Josko, 1996). However, the stressful paradigms used in these studies involved painful stimuli. Consequently, the nociceptive effects cannot be separated from the non-physical or psychological effects. That is why the controllable stress situation chosen in this study was without painful stimuli.

It is well known that thyroid hormones are very important in the normal development of vertebrate skeletal muscle (Gambke et al., 1983). All members of the myosin heavy chains multigene family respond to triiodothyronine (T_3). However, the mode of response is not intrinsic to any myosin heavy chain gene, but is determined in a highly muscle type-specific manner (Izumo et al., 1986; Li and Larsson, 1997). In the masseter superficialis of hyperthyroid animals, Izumo et al. (1986) reported that fast myosin heavy chain 2A gene expression was reduced to undetectable levels whereas it did not change significantly in hypothyroid animals. Hyperthyroidism did not cause a significant change in fast myosin heavy chain 2B gene expression in the masseter superficialis but hypothyroidism caused its deinduction (Izumo et al., 1986). In the present study, the controllable stress procedure decreased the relative expression of myosin heavy chain 2A and increased the expression of myosin heavy chain 2B in the masseter superficialis. As plasma thyroid hormones levels can be affected by a stressful situation, thyroid function could be one of the factors involved in the muscle structure and behavioural changes observed after the controllable stress procedure. The changes in myosin heavy chains isoforms observed in the masseter superficialis of stressed animals resemble those seen in hyperthyroid animals. Furthermore, the mode of response to stress seems to be determined in a highly muscle type-specific manner, since only two out of the three muscles studied modified the expression of myosin heavy chains isoforms after the stress procedure. These results are then in accordance with the previous observations of Izumo et al. (1986) and Li and Larsson (1997).

Other categories of hormones related to stress could also be involved in these modifications of muscle structure. Indeed, glucocorticoids, released during stressful situations, have catabolic effects on skeletal muscle (for review, see Lapier, 1997). These effects demonstrate fibre-type specificity: type 2B fibres are the most susceptible and type 1 fibres are the least susceptible to the atrophic effects of glucocorticoids. Falduto et al. (1990) have shown that glucocorticoid treatment causes a regional change in the myosin phenotype in a fast-twitch muscle, the plan-

taris, by increasing myosin heavy chain 2A expression only in the superficial layer. Dubois and Almon (1984) examined the glucocorticoid receptor population in skeletal muscle of the rat and observed a significantly higher concentration of sites in the slow-fibre soleus muscles than in the fast-fibre extensor digitorum longus muscle. This fibre-specific distribution could explain the muscle type-specific effects of glucocorticoids and could be at the origin of the specific effects of the stress procedure on muscles.

In this study, antidepressant treatment prevented the structural changes in masseter superficialis induced by the controllable stress procedure but was without effect in the other muscles studied. Some central effects of tricyclic antidepressants have been investigated, particularly their regulation of central glucocorticoid receptors since antidepressants increase glucocorticoid receptors in the rat hippocampus but not in the parietal cortex (Seckl and Fink, 1992). However, the relation between the peripheral glucocorticoid receptors and the effects of antidepressants is still unknown. The muscle-specific protective effect of clomipramine could then be explained by a site-specific regulation of central or peripheral glucocorticoid receptors. Furthermore, in humans, facial pain syndromes are associated with psychological stress and affect especially the masseter superficialis (Feinmann et al., 1984). Antidepressant treatment relieves pain in facial muscle disorders (Feinmann et al., 1984), which could explain the specific protective effect observed in this experiment.

Massol et al. (1990) have studied the effects of tricyclic antidepressants, including clomipramine, on thyroid function in rats in the learned helplessness paradigm. They reported that tricyclic antidepressant therapy decreased the T_3 levels in helpless and control rats. However, T_3 has been found to exert an antidepressant-like effect in various psychopharmacological tests in rodents (Brochet et al., 1987). In the stress groups of this study, the clomipramine treatment decreased the myosin heavy chain 2B content and increased the myosin heavy chain 2A content in the masseter superficialis muscle compared to the effect of saline treatment. As the same myosin heavy chains profile was observed in the masseter superficialis under hypothyroid conditions (Izumo et al., 1986), an additional explanation could be that clomipramine works by decreasing thyroid function. However, clomipramine treatment in stressed animals induced an increase in the median duration of mastication events. This lengthening was accompanied by structural modifications of the masseter superficialis, leading to a less fatigable muscle. The lower fatigability of the masseter superficialis could thus underlie the increased duration of the mastication events.

In a controllable stress situation, several factors probably affect the myosin heavy chains composition of muscles. Changes in behaviour could be one of these factors. Indeed, this study identified masticatory behaviour modifications that accompanied changes in muscle structure. Because plasma thyroid hormones levels can be affected by a stressful situation, thyroid function could then be a second factor involved in the changes in muscle structure observed after the controllable stress procedure. Glucocorticoids are also capable of changing the muscle structure in a muscle-specific manner (Falduto et al., 1990) and could therefore be a third factor. Although verification of these hypotheses requires additional experiments, clomipramine prevented stress-induced structural changes in a muscle-specific manner and could therefore act through any or all of these mechanisms. However, because the ability to control the stressor influences the consequences of stress, it would be interesting to evaluate the effects of uncontrollable bright light stress and antidepressant treatment on muscle structure and masticatory behaviour in a further study.

Acknowledgements

We thank Dr. A. D'Albis for helpful comments and suggestions, Ciba-Geigy for the generous gift of clomipramine, the Centre National d'Etudes Vétérinaires et Alimentaires, Malzéville, for lending the integration densitometer Bio-Rad GS-700 and M.M.A. Morel and C. Vogel for technical assistance. This work was supported by grants from the Centre National de la Recherche Scientifique (URA 1293).

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