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AGING: EFFECTS ON THE PROSTAGLANDIN PRODUCTION BY SKELETAL MUSCLE OF MALE RHESUS MONKEYS (*MACACA MULATTA*)*

M. K. YOUNG, R. M. BOCEK, P. T. HERRINGTON and C. H. BEATTY**

Division of Perinatal Physiology, Oregon Regional Primate Research Center, Beaverton, Oregon 97006, and Department of Biochemistry, University of Oregon Health Sciences Center, Portland, Oregon 97201 (U.S.A.)

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SUMMARY

Prostaglandin (PG) production from [1-^{14}C] arachidonic acid was measured in homogenates of sartorius muscle of aged (more than 20 years) and young adult (7 to 11 years) rhesus monkeys. Total production by the aged series (3.21 ± 0.31 (S.E.) nmol PG per g N per min) was about two times that of the young series (1.76 ± 0.14 (S.E.) nmol per g N per min). Epinephrine-stimulated PG production was also twice as great in the aged muscle series as in the young adult series (7.22 ± 0.74 (S.E.) and 3.54 ± 0.52 (S.E.) nmol per g N per min). This difference was mainly due to greater production of 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$. In both series, the addition of 500 μM epinephrine to the assay media significantly increased production of all types of PGs measured (6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE_2 plus thromboxane B_2 [TXB_2], the stable breakdown product of TXA_2, and PGD_2). The absolute increases in all types of PGs studied in the presence of epinephrine were significantly greater in the aged muscle, with the exception of $PGF_{2\alpha}$. The distribution patterns of the various types of PGs studied were similar in all series, except for 6-keto-$PGF_{1\alpha}$; this PG accounted for a greater percentage of the total production by aged muscle than by young adult muscle ($p < 0.05$).

INTRODUCTION

Recently, there has been considerable interest in the exploration of the cellular and molecular basis of the physiological changes that occur with aging. A generally accepted feature of aging populations is the progressively modified ability to adapt to an environmental challenge [1]. Sartin *et al.* [1] have presented evidence that this type of response in the liver may be the consequence of altered control of the secretion of key hormones.

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**Complete mailing address: Dr. C. H. Beatty, Division of Perinatal Physiology, Oregon Regional Primate Research Center, 505 N.W. 185th Avenue, Beaverton, Oregon 97006, U.S.A.

Many investigators have reported changes, mainly decreases, in enzyme activity in aged tissues [2, 3], whereas the activity of other enzymes remains unchanged. There is recent evidence that the accumulation of inactive enzymes is not an invariable concomitant of aging [4]. The theoretically attractive concept that some aspect of protein biosynthesis is altered with age in such a way as to produce defective proteins or an insufficient amount of specific proteins is under attack. Recent work raises the possibility that defective enzyme molecules may be produced by the action of proteases liberated during tissue extraction and are not age-related changes [5].

Prostaglandins (PGs) have been identified as mediators, via the cyclic nucleotide system, of a wide range of biological and biochemical events in mammalian tissues. Several workers have reported that the distribution patterns of the PGs change as cells age in culture [6–8]. A current theory of aging at the biochemical level is based on an increase in the production of free radicals with subsequent damage to biological systems [9]. Concurrent with the increase in free radicals is a decrease during aging in the tissue levels of antioxidants and radical quenchers. A number of steps in the production of PGs, thromboxane A_2 (TXA_2), and prostacyclin (PGI_2) from archidonic acid produce lipid peroxides and are sensitive to antioxidants [10, 11]. Therefore, it is reasonable to postulate that PG production may vary with advancing age. It has been established that substances that act as radical scavengers and reducing agents can stimulate PG biosynthesis under some conditions and depress it under others.

To date, few data are available on the amounts and types of PGs synthesized by skeletal muscle [12], and there is evidence for considerable tissue specificity in the quantitative and qualitative production of PGs by various tissues such as liver, uterus, kidney, and heart. We wished to determine whether or not changes comparable to those seen in cell culture occurred when tissues aged *in vivo*. We therefore measured the types and amounts of PGs produced by skeletal muscle homogenates from old (over 20 years of age) and young adult (7 to 11 years) rhesus monkeys and the sensitivity of the muscle to epinephrine at both ages.

MATERIALS AND METHODS

Skeletal muscle biopsy specimens were obtained from aged rhesus monkeys (*Macaca mulatta*) (over 20 years of age) and young adult rhesus (7 to 11 years old). All the monkeys were male; the aged monkeys had maintained their weight. The monkeys were anesthetized with 2-bromo-2-chloro-1,1,1-trifluoroethane (Halothane), 1% or less in a mixture of 75% oxygen and 25% nitrous oxide or *dl*-2-(*O*-chlorophenyl)-2-(methylaminocyclohexane hydrochloride (Ketalar), 5 mg/kg body weight. The sartorius and the brachioradialis muscles were excised, stripped of fat and connective tissue, rinsed in normal saline, and frozen within 5–10 min of excision in a Wollenberger clamp at liquid-nitrogen temperature. The muscle was then wrapped in aluminum foil and stored at liquid-nitrogen temperature.

Each frozen biopsy sample was cut into small pieces, homogenized in 0.1 M potassium phosphate buffer (pH 7.4) (4 °C) with a Polytron ST-20 generator for two 5-sec

intervals at three-fourths the maximum setting. The resulting 12.5% (w/v) homogenate was used immediately in the assay. Homogenates were used because of possible age-related changes in membrane permeability or uptake mechanisms in intact cells. Analyses were done concurrently on young adult and aged muscle homogenates. The homogenate (0.6 ml) was incubated in 0.1 M potassium phosphate buffer (pH 7.4) (37 °C) with or without 0.5 M epinephrine in a final volume of 1.0 ml. After a 10-min preincubation in a Dubnoff shaker, the reaction was initiated with the addition of 7.5 nmol (0.35 μCi) of [1-^{14}C] arachidonic acid (47 mCi/mmol, New England Nuclear) and was terminated after 20 min by the addition of 0.2 ml of 0.5 M citric acid. Boiled tissue blanks were run concomitantly. The homogenates were extracted twice with 2.0 ml of freshly distilled ethyl acetate. Ten micrograms of authentic PG standards (6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE_2, and PGD_2) were added, and the ethyl acetate extract was evaporated under nitrogen at 37 °C in a water bath. The residue was redissolved in 50 μl of ethyl acetate and spotted on a silica gel G, 250-μm thin-layer chromatography (TLC) plate (Analtech). Each plate was developed in the organic phase of an ethyl acetate–isooctane–acetic acid–water mixture (11:5:2:10), and the PG standards were visualized with iodine vapor. With this technique it was not possible to separate PGE_2 and TXB_2. However, these two compounds were separated when the TLC plates were developed in 1% acetic acid–ethyl acetate [13]. We assumed that the activity remaining at the origin was phospholipid as described by Murota and Morita [14]. Areas corresponding to the authentic standards were scraped into scintillation vials and counted in a Packard scintillation counter. Recovery from the TLC plates of the initial activity added to homogenates averaged 55%. Recovery from the TLC spot of [^{3}H]$PGF_{2\alpha}$ added to the homogenate averaged 75%. Berlin *et al.* [12] reported a 73% recovery. The concentrations (per min per g N) of PGs were calculated from the specific activity of the arachidonic acid substrate corrected for the percentage of total counts added to each assay that was recovered on TLC and for the blank values found for the heat-inactivated enzyme.

Prostaglandin production and incorporation of [^{14}C] arachidonic acid into phospholipid were linear with time (5 to 25 min incubation) and enzyme concentration (1 to 4 mg of nitrogen per assay). In some experiments an unknown radioactive metabolite of labeled arachidonic acid migrated with the $PGF_{2\alpha}$. This metabolite could be distinguished on the TLC plate as a thin pigmented line. We separated it from the $PGF_{2\alpha}$ by developing the plates twice.

Nitrogen values were determined on a Kjeldahl digest with a Technicon Autoanalyzer. The DNA concentrations were assayed by the method of Halprin *et al.* [15]. No differences were considered significant unless the p value was < 0.05.

RESULTS

Total basal PG production in the predominantly red sartorius muscle from aged rhesus macaques was almost twice that in the sartorius from young adults (Table I). This difference was mainly due to the greater production of the stable metabolite of PGI_2, 6-keto-$PGF_{1\alpha}$, and $PGF_{2\alpha}$. Each of these PGs accounted for 30 to 40% of the total PG pro-

TABLE I

PROSTAGLANDIN PRODUCTION AND INCORPORATION OF [^{14}C] ARACHIDONIC ACID INTO PHOSPHOLIPID BY HOMOGENATES OF SKELETAL MUSCLE FROM AGED AND YOUNG ADULT MALE RHESUS MONKEYS

	nmol prostaglandins produced per g/N per min.		
Series	*Aged (A)*	*Young adult (B)*	*p (A vs. B)*
6-keto-$PGF_{1\alpha}$ basal	1.19 ± 0.13	0.52 ± 0.12	< 0.01
+ epinephrine	3.10 ± 0.39	1.14 ± 0.30	< 0.005
$PGF_{2\alpha}$ basal	1.24 ± 0.19	0.63 ± 0.09	< 0.025
+ epinephrine	1.92 ± 0.23	1.20 ± 0.11	< 0.05
PGE_2 + TXB_2 basal	0.62 ± 0.15	0.42 ± 0.06	> 0.10
+ epinephrine	1.77 ± 0.33	0.90 ± 0.17	< 0.05
PGD_2 basal	0.16 ± 0.02	0.19 ± 0.03	> 0.10
+ epinephrine	0.43 ± 0.07	0.30 ± 0.04	> 0.10
Total prostaglandins	3.21 ± 0.34	1.76 ± 0.14	< 0.005
+ epinephrine	7.22 ± 0.74	3.54 ± 0.52	< 0.01
Phospholipids basal	15.9 ± 1.9	9.39 ± 1.03	< 0.02
+ epinephrine	14.0 ± 1.4*	8.96 ± 0.91	< 0.02

Values are means ± S.E.M., six monkeys in each series (duplicate analyses). *p* values were always < 0.01 for the effect of adding 500 μM epinephrine on prostaglandin production (statistical analyses on the basis of paired samples). Homogenates of muscle from aged and young adult monkeys were incubated and run on TLC plates simultaneously.

*p value for decrease in [^{14}C] arachidonic acid incorporation into phospholipid with epinephrine < 0.05, statistical analyses on the basis of paired analyses.

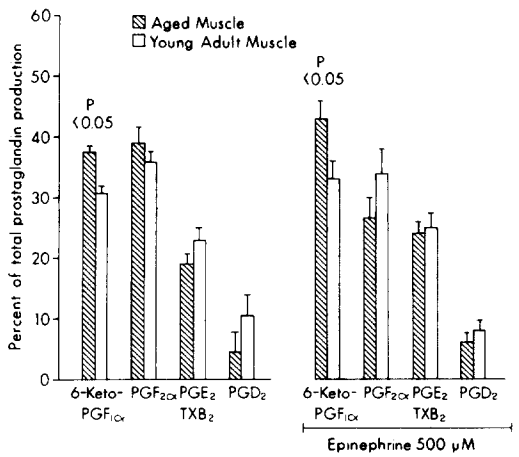

Fig. 1. Effects of age on the percentage distribution of prostaglandins (PGs) produced by homogenates of sartorius muscle from aged (over 20 years) and young adult (7 to 11 years) male rhesus monkeys incubated with and without 500 μM epinephrine. The vertical bars represent means with one S.E.M. The *p* values for the differences between the production of 6-keto-$PGF_{1\alpha}$ by aged and young adult muscle are indicated above the bars.

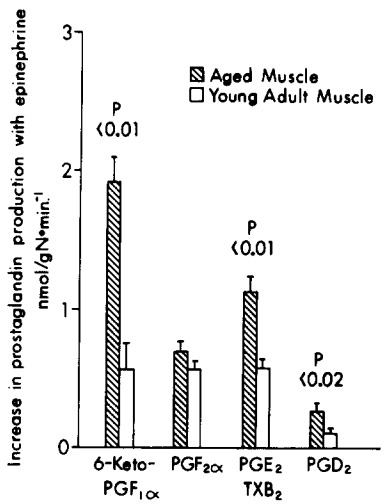

Fig. 2. Effects of 500 μM epinephrine on the prostaglandin (PG) production of homogenates of sartorius muscle from aged and young adult rhesus monkeys. The vertical bars represent means with one S.E.M. The p values for the difference between the two series are indicated above the bars. The p values for the addition of epinephrine are all < 0.01 (paired analysis).

duction (Fig. 1). Although the total production was greater in aged than in young adult muscle, the distribution patterns of the various PGs for the two series were quite similar, except that for 6-keto-$PGF_{1\alpha}$. The percentage of total production accounted for by 6-keto-$PGF_{1\alpha}$ was significantly less in young adult than in aged muscle. There were no differences in the two series in the distributions of the other PGs. Incorporation of [1-^{14}C]-arachidonic acid into the phospholipid fraction was greater in the aged series (Table I).

The addition of 500 μM epinephrine to the assay media in both series significantly increased production of all types of PGs studied (Table I). Although the overall distribution patterns for the different PGs remained quite similar in young adult and aged muscle in the presence of epinephrine (Fig. 1), the absolute increases (pmol per g N per min) in the production of the different types of PGs studied were greater in the aged series, except for $PGF_{2\alpha}$ (Fig. 2). Epinephrine caused a slight (12%) decrease in [^{14}C] arachidonic acid incorporation into phospholipid aged muscle but had no effect on incorporation into phospholipid in young adult muscle (Table I). The incorporation of [^{14}C]arachidonic acid into phospholipids was 36 to 40% greater in the aged series with and without epinephrine.

production by homogenates of the predominantly red area of the brachioradialis muscle from adult macaques by developing the TLC plates in 1% acetic acid–ethyl acetate. An average of 72% of the sum of both PGs produced was PGE_2 and 28% was TXB_2, with and without epinephrine. From the results of these experiments we calculated that PGE_2 represented about 17% and TXB_2 about 6% of the total PG production, both with and without epinephrine. Epinephrine doubled the production of both PGs. A number of workers [16–18] have included glutathione (GSH) in their PG assay medium. However,

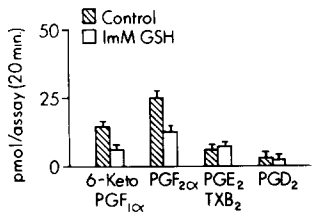

Fig. 3. Effect of 1 mM glutathione (GSH) on the prostaglandin (PG) production of adult rhesus sartorius muscle. Values are averages of two experiments (duplicate analyses).

TABLE II

COMPARISON OF DRY WEIGHTS, NITROGEN CONTENT AND DNA LEVELS OF SARTORIUS MUSCLES FROM AGED AND YOUNG ADULT MALE RHESUS MONKEYS

	Young adult	*Aged*	*p*
Percentage dry weight	23.6 ± 0.6 (7)	22.2 ± 0.4 (7)	> 0.10
Nitrogen (mg N per g wet wt.)	30.5 ± 0.6 (6)	30.0 ± 0.5 (6)	> 0.10
DNA (mg per 100 g wet wt.)	48.8 ± 1.7 (7)	57.0 ± 2.0 (10)	< 0.01

Values are means ± S.E.M.; numbers in parentheses are number of monkeys, duplicate analyses.

with homogenates of adult sartorius, GSH inhibited production of 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ (Fig. 3) and therefore we did not use this compound in our assay.

There were no differences in the nitrogen concentrations and the dry weights of young adult and aged sartorius (Table II). Therefore, the difference in PG production cannot be explained by a difference in the base of reference. Since the DNA level changed with age, *i.e.*, the DNA was 17% higher in the aged muscle, this compound is not suitable for use as a base of reference [2].

DISCUSSION

Prostaglandins are thought to be ubiquitous in mammalian tissues and to have a wide range of biological and biochemical actions. It is therefore reasonable that the metabolism of these compounds varies during growth and development, maturity, and senescence. We are studying the changes in PG metabolism that occur in skeletal muscle during aging of nonhuman primates. Skeletal muscle is a large tissue that represents 40 to 45% of the total body weight in adult human beings and rhesus monkeys [19]. By virtue of its mass, therefore, the metabolism of this tissue is of major importance to the total body economy. Atrophy occurs in muscle with advanced age. However, the clinical condition

of our monkeys at the time of biopsy indicated that the metabolic changes appeared early in senescence or at a "presenile" stage.

Our results on adult rhesus muscle differ somewhat from those of Berlin *et al.* [12] on human muscle. They reported that PGE_2 constituted over 50% of the total [^{14}C]PG activity formed from [^{14}C] arachidonic acid by aged human muscle, but they sampled no young adult muscle. In our experiments with rhesus muscle, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ were the major PGs synthesized, and PGE_2 represented about 15% of the total PG production. Berlin *et al.* [12] obtained biopsy specimens from six patients 50 to 73 years old during operations for obliterative arteriosclerotic disease and assayed a 4000 *g* supernatant of muscle homogenates. Species difference may also explain the differences in our results.

With rhesus monkeys, total PG production by skeletal muscle was greater in the aged than in the young adult series, both in the basal state and after epinephrine stimulation. The greatest difference was in the 6-keto-$PGF_{1\alpha}$ fraction. Twice as much 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ was synthesized by aged muscle in both the basal and epinephrine-stimulated series. These results on *in vivo* aging are different from the results of others on cultured cells. Chang *et al.* [6] reported that aortic smooth muscle cells from young rats in culture produced more 6-keto-$PGF_{1\alpha}$ than cells from old rats. Additional data on cultured lung fibroblasts strongly suggest that the synthesis of 6-keto-$PGF_{1\alpha}$ decreases with *in vitro* aging [7, 8].

Murota and Morita [14] studied aging and PG synthesis in rat liver and reported a 60% decrease in synthesis from 72 to 90 weeks of age, an effect opposite to that seen in aging skeletal muscle. Incorporation of arachidonic acid into phospholipids increased with age in both rat liver and rhesus muscle.

Alterations in sensitivity to hormones are characteristic of aging organisms [1, 20] and may reflect either increased or decreased sensitivity to the stimuli. The sensitivity of PG endoperoxide synthetase in macaque muscle to stimulation by epinephrine was clearly greater in the aged than in the young adult series. Although most studies show decreased receptor concentrations during senescence, in some instances they may increase [20]. Murota and Morita [14] reported a decrease in the sensitivity of PG synthesis to stimulation with aging.

One current theory of biochemical aging is based on the production of free radicals and subsequent damage to biological systems [9]. Lipid peroxides are produced during the enzymatic conversion of arachidonic acid to PGs, TXs, and PGI_2. A number of steps in the arachidonate-dependent PG pathway are vulnerable to antioxidant effects. Such points in the biosynthetic sequence include PG endoperoxide synthetase, both the cyclooxygenase and peroxidase activity, PGI_2 synthetase, TX synthetase, and lipoxygenase.

Compounds that act as radical scavengers and reducing agents can affect PG biosynthesis *in vivo* and *in vitro*; whether they increase or decrease it depends on the experimental conditions [10, 11]. Egan *et al.* [11] hypothesize that the levels of endogenous radical scavengers vary widely among cell types. The effects of aging on the production of PGs *in vivo* might therefore vary among different tissues, and the differences might depend on the endogenous concentrations of radical scavengers and reducing agents. The

addition of the reducing agent glutathione (GSH) to our assay medium inhibited the formation of 6-keto-$PGF_{1\alpha}$ by adult muscle. Cottee *et al.* [16] reported similar results with ram seminal vesicle microsomes. One can postulate therefore that the level of reducing agents in these preparations is low.

The 17% increase in DNA concentrations per unit wet weight in our aged macaque muscle agrees with the results reported by Steinhagen-Thiessen and Hilz [21]. They reported more DNA in human striated muscle obtained in surgery from elderly patients (64 to 84 years) than in muscle from a younger group (24 to 47 years), and they felt that this age-related increase in DNA content was probably due to a loss of tissue water. Gutman [22] reported that the water content of senescent rat muscle was decreased. This explanation does not apply to our macaque muscle since the percentage dry weight and the nitrogen levels were similar in the aged and young adult series. However, one could theorize that with aging there had been a decrease in the volume of the white fibers [22, 23], so that there were more fibers per unit area and therefore no change in the nitrogen content per gram wet weight. This could explain an increase in DNA content. Presumably the number of nuclei (the DNA) per muscle fiber and per whole muscle remained the same while the DNA per gram of muscle increased. Preliminary data in our laboratory have indicated that the DNA content of some rhesus muscles reached its maximum value by 2 years of age whereas in other muscles the total DNA content is still increasing rapidly at this age (unpublished data). In mice there appears to be an age-dependent decrease in the ratio of DNA to protein in all subcellular fractions of muscle [22].

This study on PG production by muscle from male rhesus monkeys should also be done on females. Gecse *et al.* [24] have demonstrated a marked sex difference in PG metabolism; the formation of PGE_2, $PGF_{2\alpha}$, and PGD_2 from arachidonic acid by microsomal preparations from kidney medullas of female rats was significantly less than that by preparations from male rats.

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