Psychosocial stress causes endothelial injury in cynomolgus monkeys via $\beta_1$-adrenoceptor activation

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Abstract

Current evidence links psychosocial factors to exacerbation of diet-induced atherosclerosis in monkeys via activation of the sympathetic nervous system. However, it is uncertain whether these factors can potentiate initial lesion formation, and do so even in the absence of dietary provocation, and whether any such effects can be prevented by beta-adrenergic blockade. As endothelial injury has been considered an initiating event in atherogenesis, we studied the effect of psychosocial stress on endothelial integrity in 48 adult male cynomolgus monkeys (Macaca fascicularis). All animals were housed in 12 social groups of four monkeys each for 11 weeks. The monkeys in half of the groups were exposed to a socially unstable (‘stressed’) condition for 72 h and received saline ($n=8$), a lipophilic $\beta_1$-blocker (metoprolol, 0.30 mg/kg per h; $n=8$), or hydrophillic $\beta_1$-blocker (atenolol, 0.15 mg/kg per h; $n=8$). The remaining six social groups were assigned to the socially stable (non-stressed) condition; for 72 h these animals all remained in their social groups and were similarly treated with saline ($n=8$), metoprolol ($n=8$), or atenolol ($n=8$). The frequency of IgG-positive (injured) endothelial cells was estimated on en face (Häutchen) preparations from the thoracic aorta and coronary arteries. Psychosocial stress caused a significant increase in the number of injured endothelial cells in the circumostial areas of the descending thoracic aorta in the placebo group (0.3 vs. 0.8%, $P<0.02$), an effect that had not been demonstrated previously. Moreover, $\beta$-blockade significantly ($P<0.01$) inhibited the stress effect, with no differences between the two beta-blocking agents. The number of injured endothelial cells in the non-branched portions of the aorta and coronary arteries were low and indistinguishable among groups; irregularities in the size and location of branching points in the coronary arteries precluded analysis of these sites. This study demonstrated that psychosocial stress induces endothelial injury, and that this effect is mediated via $\beta_1$-adrenoceptor activation. © 1998 Elsevier Science Ireland Ltd.

Keywords: Atherosclerosis; Endothelial injury; Leukocyte adhesion; Häutchen preparation; Sympathetic activation; Cynomolgus macaque

1. Introduction

Epidemiological evidence suggests that psychosocial stress is an independent risk factor in the development of atherosclerosis [1]. Experimentally, the stress associated with an unstable social environment causes significant exacerbation of coronary artery atherosclerosis in cholesterol-fed cynomolgus monkeys (Macaca fascicularis) that are habitually dominant in their social groups [2]. The adverse effect of stress on atherosclerosis development in such animals can be inhibited with
β-blockade, suggesting that the behavioral influences on atherosclerosis are mediated by activation of the sympathetic nervous system [3]. The current study considers whether stress-induced sympathetic activation similarly causes endothelial injury in the absence of dietary provocation, whether such effects extend to the coronary arteries, and whether any inhibitory effects of beta-adrenergic blockade relate to the lipophilicity of the agent.

Endothelial injury is thought to be an initiating event in atherogenesis [4–6], and one of the hallmarks of irreversibly injured endothelial cells is the presence of intracellular IgG [7,8]. Such IgG-containing cells can be visualized immunohistochemically [7,9] and quantitated on en face (Hautchen) preparations. Several studies have confirmed the association of IgG-containing cells with other criteria of irreversible endothelial injury, such as the presence of calcium deposits [10], ultrastructural deformations [7,8] and staining for uncomplexed Evans-Blue [7]. IgG-positive cells in Hautchen preparations have also been shown to be co-distributed with replicating endothelial cells in rat aorta [11] and rabbit aorta (Pettersson et al. unpublished data), suggesting that irreversible endothelial injury triggers neighboring cells to replicate. This finding was confirmed also in the thoracic aorta of cynomolgus monkeys [9].

We have shown previously that pharmacologic induction of sympathetic activation with chloralose causes significant endothelial injury in the thoracic aorta of rabbits, which can be prevented by β-blockade [12]. A preliminary study also demonstrated that β-blockade, with a lipophilic adrenoceptor antagonist, reduces endothelial injury in circumostial areas of the descending thoracic aorta of cynomolgus monkeys subjected to acute social disruption [9]. In this initial study, however, there was no control (non-stressed) group, precluding any statement about the specific contribution of psychosocial stress, or the amelioration of stress-related injury by β-blockade. Moreover, repeated intramuscular injections of radiolabeled thymidine in this study required excessive physical manipulation of the animals, potentially overwhelming any effects of social disruption and thus further confounding any interpretation of the resulting data. Finally, the animals in this preliminary investigation consumed a cholesterol-containing diet for several months prior to the assessment of their arteries, preventing any conclusions concerning the independent role of non-dietary factors in the initiation of endothelial injury. Although the monkeys in the preliminary study did not have high levels of plasma cholesterol, these levels were significantly higher than the plasma cholesterol levels of the chow-fed monkeys in this study.

This study in cynomolgus monkeys was designed to determine whether psychosocial stress could cause aortic endothelial injury in the absence of dietary cholesterol, and in turn, if this effect was mediated by sympathetic activation. We also wanted to extend the Hautchen technique to the coronary arteries; and to determine whether lipophilic and hydrophilic β-blocking agents (metoprolol and atenolol, respectively) might be equally effective in preventing stress-induced arterial injury. Cynomolgus monkeys are appropriate subjects for such studies because these animals can be manipulated in ways that induce psychosocial stress [2], and there are similarities between these animals and human beings in the development and distribution of atherosclerotic plaques [13]. In a 2 × 3 factorial design, animals were assigned to either a socially stressed or unstressed condition, and within each social condition administered either saline or one of the two β-blocking agents.

2. Materials and methods

2.1. Animals

The subjects used were 48 adult cynomolgus monkeys (Macaca fascicularis) imported from Indonesia. These animals varied from 7.5 to 10.5 years of age (estimated from dentition) and weighed between 5 and 6 kg. Before the study, all monkeys were kept in quarantine for 90 days, during which time they were given antibiotics (Baytrill, Miles, West Haven, CT). A non-atherogenic diet (Purina, St Louis, MO) was fed throughout the study; as a result, total plasma cholesterol concentrations, determined by [14], averaged 103 ± 2 mg/dl across groups. All experimental procedures were conducted in accordance with state and federal guidelines and with the approval of our institution’s animal care and use committee (IACUC). The Bowman Gray School of Medicine is fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC).

2.2. Experimental procedure

2.2.1. Social manipulation

Baseline period: the monkeys were allocated to 12 social groups of four animals each. Each group was maintained in a stable social condition for 11 weeks.

Experimental period: following completion of the baseline period, each group of animals was randomly assigned to one of six cells in a 2 × 3 factor design: (Conditionstress, no-stress × Treatmentplacebo, atenolol, metoprolol). There were thus eight animals (two social groups) randomly assigned to each experimental condition. The stress condition was comprised of a 3-day (72 h) procedure in which animals were taken out of their baseline social groups and each placed in a ‘host’ social group of four male monkeys that had just been formed. There
were four such host groups (16 monkeys) that otherwise were not part of the experiment. The provision of four host groups allowed an entire experimental group (n = 4 animals) to be subjected to the stress procedure at the same time. Thus, on day 1 of the manipulation, each of the four animals in a group assigned to the stress condition was placed in one of the four host groups. These ‘target’ animals stayed in the host groups for 72 h. The host groups were themselves reconstituted by random rearrangement among the 16 host animals just prior to the beginning of each 72 h manipulation. Animals assigned to the stable (non-stressed) social condition simply remained in their original groups throughout the 72 h experimental period. The rationale for this manipulation is that cynomolgus monkeys, when living in social groups, form stable dominance hierarchies as well as establish alliances and friendships. We have shown previously that disruption of these relationships by exposure of animals to new social groups induces psychosocial stress, as evidenced by an increase in the frequency and intensity of antagonistic encounters among group members [2,9]. An experimental period of 72 h was chosen to insure that any ensuing arterial damage represented lesion initiation and not lesion progression.

2.2.2. Heart rate measurements

Elevations in heart rate have been used as a marker for arousal of the sympathetic nervous system in cynomolgus monkeys [3]. Thus, heart rate was recorded in the present experiment in order to evaluate the effectiveness of the psychosocial stressor. Immediately prior to the experimental period, each monkey was fitted with a portable electrocardiogram telemetry transmitter unit (LSE, Tullahoma, TN), which was attached beneath a nylon mesh jacket. Baseline heart rates were recorded sequentially during 2 × 24 h periods, at the end of the 11-week baseline period. Continuous heart rate recordings were made during the sampling sequences, which lasted 0.5 min every 2–4 min, depending on whether four or eight monkeys were sampled at the same time. Following the baseline heart rate period, experimental period heart rates were recorded during 2 × 24 h sessions, using the same sampling sequence and duration.

2.2.3. Pharmacological manipulation and analysis

Before introducing the animals to the assigned social treatments for the experimental period, they were anesthetized with ketamine hydrochloride and samples of blood and cerebrospinal fluid (CSF) were obtained for measurements of baseline drug level concentrations. Next, each animal was implanted with an osmotic minipump (Alzet 2ML1, Alza, Palo Alto, CA) filled with saline, atenolol or metoprolol as specified for the animal’s treatment group. The pumps delivered 0.15 mg atenolol/kg per h or metoprolol 0.30 mg/kg per h.

The analytical methods used for determination of drug concentrations in plasma and CSF were: (1) reversed-phase liquid chromatography and fluorometric detection for atenolol; and (2) high-resolution gas chromatography and electron-capture detection for metoprolol [15].

2.2.4. Necropsy

The monkeys were deeply anesthetized with ketamine hydrochloride (30 mg/kg i.m.) and sodium pentobarbital (1 mg/kg i.v.), after which blood and CSF samples to determine drug concentrations during the experimental period were collected. Following this, sodium heparin (250 IU/kg i.v.) was given, and the thorax was opened. The vascular system was rinsed first with Dulbecco’s phosphate buffer (pH 7.4) and then fixed with 4% neutral phosphate-buffered formaldehyde. The pressure of the influx perfusates was kept constant at 100 mmHg. After fixation, the heart and the thoracic portion of the aorta were removed and carefully placed in a large bowl with Dulbecco’s buffer. During all handling of the aorta and the heart, care was taken not to apply any mechanical force to the tissues that could injure the endothelium.

2.3. Tissue preparation

2.3.1. Descending thoracic aorta

The adventitia of the aorta was removed, and the vessel was cut into five or six segments. The most proximal segment that lacked intercostal artery orifices was used for antibody control incubations. The following segments usually contained two pairs of intercostal artery orifices each. The segments were placed on a piece of teflon, after which they were opened along the ventral aspect and pinned flat.

2.3.2. Coronary arteries

The heart was separated from the aorta, and the epicardial parts of the left anterior descending (LAD) and left circumflex (LCX) coronary arteries were dissected out and placed in a dish with Tris–phosphate buffered saline (PBS). LAD and LCX were then dissected free of fat and cut into two and three segments, respectively, opened longitudinally, and pinned flat on a piece of teflon.

2.4. Immunohistochemistry

2.4.1. Identification of injured endothelial cells

The segments were incubated with F(ab')2 fragment of goat anti-monkey IgG (protein concentration 8 mg/ml, dilution 1:200, Cappel, Durham, NC), as a primary antibody. After 30 min, the segments were rinsed 3 × 10
min with Tris–PBS and the secondary antibody, peroxidase-conjugated affinity-purified F(\(ab'\))\(_2\) fragment of rabbit anti-goat IgG (protein concentration 2.44 mg/ml, dilution 1:200, Cappel, Durham, NC), was applied. The incubation time and the rinsing procedure were the same as for the primary antibody.

To control the specificity of the antibodies, the following two incubations were performed: (1) for control of the primary antibody, the F(\(ab'\))\(_2\) fragment of goat anti-monkey IgG was replaced with non-immune goat IgG (Cappel, Durham, NC), with the same protein concentration as the primary antibody; (2) for control of the secondary antibody, the incubation with the primary antibody was omitted.

The segments were developed for peroxidase activity with 3,5'-diaminobenzidine (1 mg/ml, Sigma, St. Louis, MO) containing 0.02% H\(_2\)O\(_2\) as final concentration.

2.5. Häutchen preparation

The procedure of the Häutchen technique for the thoracic aorta has been described previously [9,12]. Briefly, the segments were dehydrated in 35, 70, 95 and 2 \(\times\) 100% ethanol (15 min in each bath). The needles were removed and fine forceps were used to grasp one corner of the segment. The endothelial surface was allowed to air dry for a few seconds and it was then coated with 1–2 drops of ether–ethanol (5:1) and immediately placed with the endothelial side down on a glass slide, previously covered with nitrocellulose. The segment was pressed with absorbent paper for 20 s. The segments were rehydrated in 35% ethanol for 30 min, after which the adventitia and media layers were removed leaving the endothelium attached to the nitrocellulose. The entire nitrocellulose film was then detached from the glass slide and after a short dip in 35% ethanol, the film was remounted on the slide, now with the luminal side up. The endothelium-nitrocellulose was covered with a square a fine plastic net (Derma AB, Gråbo, Sweden) and secured over the slide with a pair of plastic artery clips. The nitrocellulose was then dissolved in ether–ethanol (4:1) for 10 min after which the tissue was allowed to air dry. Mayer’s Hematoxyline (Apteksbolaget, Gothenburg, Sweden) was used to counterstain the nuclei.

2.6. Identification of adherent leukocytes

Adherent cells, probably leukocytes, were identified by their morphological appearance. They differed from the endothelial cells and smooth muscle cells by their size, shape and the intensity of the hematoxyline staining.

2.7. Estimation of endothelial injury

Preliminary examination of the Häutchen preparations indicated a positive correlation between the numbers of adherent leukocytes and injured endothelial cells. In order to avoid a potential confound due to the presence of various amounts of adherent leukocytes and to obtain a relative leukocyte-independent estimate of endothelial injury, we only counted injured endothelial cells in visual fields which met one of the following criteria: (1) in non-branched areas with \(\leq\) 26 leukocytes/visual field (\(\times\) 250 magnification); or (2) in circumostial areas of the descending thoracic aorta with \(\leq\) 65 leukocytes/visual field (\(\times\) 100 magnification). The differences in acceptable maximal amounts of leukocytes/visual field in the non-branched and circumostial areas reflect the different amounts of endothelial cells encompassed within the visual fields at \(\times\) 250 and \(\times\) 100 magnification [24].

To count the IgG-positive cells in a time-effective manner, we defined the cell count of each visual field as belonging to a grouping of cells instead of counting each cell of interest. We have previously described the advantages of the grouping procedure and the high correlation between results obtained by grouping the cell count data and exact counting [16].

2.7.1. Aorta

Injured endothelial cells were identified by their distinct brown color, which was produced by the peroxidase activity of the specific conjugated antibodies that were directed to intracellular IgG. In the descending thoracic aorta we counted injured endothelial cells in areas defined as non-branched and circumostial areas separately as these are atherosclerosis resistant and atherosclerosis prone areas. Accordingly, the IgG-positive cells in the Häutchen preparations form the non-branched area of the descending thoracic aorta were counted through an eyepiece with a grid at \(\times\) 250 magnification. Starting from a standardized distance from the edge of the Häutchen preparation, the IgG-positive cell counts were estimated and expressed as a percentage of the total number of endothelial cells within the visual field. The surface area corresponding to at least 100 000 endothelial cells in the non-branched areas of the descending thoracic aorta within each monkey was investigated and care was taken not to include the area surrounding the intercostal artery orifices.

The circumostial aorta was defined as the area described by a circle, with a radius of 500 \(\mu\)m, and the origin coincident with the center of the orifice. The IgG-positive cells were estimated at \(\times\) 100 magnification and expressed as a percentage of the total number of endothelial cells within the circle.
Table 1
Mean values ± S.E.M. of 2 × 24 h sequential heart rate recordings during baseline and experimental periods

<table>
<thead>
<tr>
<th></th>
<th>Placebo stress (n = 7)</th>
<th>Placebo no-stress (n = 7)</th>
<th>Metoprolol stress (n = 8)</th>
<th>Metoprolol no-stress (n = 8)</th>
<th>Atenolol stress (n = 8)</th>
<th>Atenolol no-stress (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>113 ± 6*</td>
<td>110 ± 5</td>
<td>125 ± 4*</td>
<td>109 ± 5</td>
<td>125 ± 6*</td>
<td>102 ± 3</td>
</tr>
<tr>
<td>Experimental</td>
<td>127 ± 6**</td>
<td>119 ± 4***</td>
<td>110 ± 3**</td>
<td>93 ± 4</td>
<td>111 ± 6**</td>
<td>85 ± 3</td>
</tr>
</tbody>
</table>

* P<0.001 vs. within-group baseline values by analysis of variance (ANOVA).
** P<0.015 vs. corresponding value for non-stress group by analysis of covariance (ANCOVA).
*** P<0.0001 vs. corresponding values for groups treated with β-blockers by analysis of covariance (ANCOVA).

2.7.2. Coronary arteries
The non-branched areas of the coronary artery segments were investigated through an eyepiece with a grid at ×400 magnification. As many endothelial cells as possible were screened within these segments, omitting only the areas close to the edge of the segment and those encompassing artery orifices. The irregular size and location of coronary artery orifices prevented effective a priori preparation of the vessels in a manner comparable to that used in the descending thoracic aorta. Hence, only qualitative evaluation of the circumostial regions in these arteries was possible.

2.8. Statistical analysis

2.8.1. Endothelial injury
As we have previously described, the statistical distribution of injured endothelial cell counts within a visual field is log normal and, consequently, log transformation of the data has been used [16]. To calculate the percentage of IgG-positive cells for each animal, the geometric means of the visual fields were divided by the total number of endothelial cells per visual field. The data are reported as geometric means and 95% confidence intervals.

2.8.2. Heart rate
The statistical analysis of heart rate values was based on 24 h averages (corresponding analyses based on peak hours gave similar results). In order to utilize all available information, missing values (single values, parts of 24 h periods or complete 24 h periods) were replaced using standard missing value technique [17]. The average across the two baseline periods and across the two experimental periods was used in the subsequent analysis.

The heart rate data and the total plasma cholesterol and drug concentrations are reported as mean ± S.E.M. P-values < 0.05 are considered statistically significant.

3. Results

3.1. Antemortem determinations

3.1.1. Plasma/CSF levels of drugs
At the end of the experimental period the concentration of metoprolol (n = 16) in plasma was 492 ± 28 nmol/l and in CSF 457 ± 25 nmol/l and the concentration of atenolol (n = 16) in plasma 2654 ± 180 nmol/l and in CSF 341 ± 31 nmol/l. The differences in CSF concentrations of metoprolol and atenolol reflect the impediment of the blood/brain barrier to hydrophilic substances. Metoprolol is lipophilic and easily crosses the blood-brain barrier, i.e. with a drug-distribution of 1:1 between blood and CSF compared to atenolol which is hydrophilic, and with a drug-distribution of approximately 8:1 between blood and CSF.

3.1.2. Heart rate
The heart rate data are shown in Table 1. Despite randomization, the stressed groups had significantly (P = 0.001) higher baseline heart rate values than the non-stressed groups. Therefore, baseline heart rate values were used as covariates in the subsequent analysis. Two-way analysis of covariance applied to the experimental data showed heart rates of stressed animals to be significantly higher than among unstressed controls, across all groups (P = 0.015). Overall, heart rate was reduced significantly (P = 0.0001) in the metoprolol- and atenolol-treated groups, compared to the groups not treated with β-blockers. However, the stress-by-treatment interaction term was not significant (P = 0.83).

3.2. Postmortem determinations
Results are based on 46 monkeys from the initial 48. One monkey from the stress placebo and one from the non-stress placebo conditions were excluded due to illness and a technical problem, respectively. A
Fig. 1. Häutchen preparations from aortic and coronary artery endothelium. (a) IgG-containing irreversibly injured endothelial cells were identified by their intensive brown color, which was produced by the peroxidase-conjugated antibodies directed to intracellular IgG. Calibration bar = 20 μm. (b), (c) Häutchen preparations from the descending thoracic aorta. Figures show circumostial area with an intercostal artery ostia in the centre of the figures. Significantly more injured endothelial cells were found in the circumostial areas of the descending thoracic aorta of the monkeys belonging to the stressed placebo group (Fig. 1b) than in the monkeys belonging to the corresponding non-stressed group (Fig. 1c). The arrow indicates the blood flow direction. Calibration bar = 200 μm. (d) Häutchen preparation of the coronary artery endothelium (LAD). Small branching vessel orifices were present on the coronary segments and a considerable number of injured endothelial cells appeared to be accumulated to these circumostial areas. Calibration bar = 100 μm.

more thorough description of the appearance of the Häutchen and the adhesion of leukocytes to the endothelium of the monkeys from the unstressed placebo group is published elsewhere [24].

The frequency of injured endothelial cells was estimated on Häutchen preparations from the non-branched and circumostial areas of the descending thoracic aorta (Fig. 1a). In the placebo condition, endothelial injury in circumostial areas of the descending thoracic aorta was significantly greater ($P < 0.02$) in stressed animals than among unstressed controls (Fig. 1b, c and Fig. 2a). The results in the metoprolol and atenolol groups were similar in both the circumostial areas and non-branched areas of the descending thoracic aorta. Data from these groups were therefore pooled when compared to the placebo groups. A subsequent ANOVA applied to the data from the β-blocked groups and the placebo groups ($2 \times 2$ table) indicated a significant ($P < 0.01$) interaction between psychosocial stress and β-blockade in circumostial areas of descending thoracic aorta (Fig. 2a). The results from the non-branched areas of the descending thoracic aorta demonstrated a low frequency of endothelial injury and were within the same range across treatment groups (Fig. 2b).

Injured endothelial cells in LAD and LCX were counted at non-branched sites, i.e. remote from the small branching vessel orifices which were present in these arteries. Few scattered IgG-positive cells were found at these non-branched sites; as in the aorta,
inspection of the data revealed that all treatment groups were similar (Fig. 2c). The irregular occurrence and size of the orifices prevented quantitation of branching sites in the coronary arteries. Nonetheless, a considerable number of injured endothelial cells appeared to be located at these sites (Fig. 1d).

4. Discussion

This study in cynomolgus monkeys demonstrates, for the first time, that psychosocial stress induces endothelial injury, and that this effect is mediated via $\beta_1$-adrenoceptor activation. Psychosocial stress was induced by exposing experimental animals to a novel (unstable) grouping of monkeys (socially strangers to the experimental animals) for 72 h. Corresponding non-stressed groups were used as controls. Frequent sequential heart rate recordings made during the $2 \times 24$ h baseline and experimental periods provided evidence that the behavioral manipulation was stressful and that the sympathetic component of this stress was ameliorated by $\beta$-adrenergic blockade, irrespective of lipophilicity. These results are consistent with previous research in rabbits indicating that pharmacologic activation of the sympathetic nervous system is similarly associated with aortic endothelial injury, which can be prevented by $\beta$-blockade [12]. The results are also consistent with an earlier, uncontrolled (with respect to psychosocial stress) study in monkeys showing that circumostial areas of the thoracic aorta are predilection sites for endothelial injury, compared to the non-branched areas [9]. However, the current results extend these earlier findings in at least three ways: firstly, by showing that psychosocial stress independently causes endothelial injury, probably via $\beta$-adrenoceptor activation; secondly, by demonstrating that such injury occurs in the absence of stimulation by dietary cholesterol; and thirdly, by showing that beta-adrenoceptor blockade, whether lipophilic or hydrophilic, inhibits stress-induced endothelial injury.

An intact endothelium is crucial for the maintenance of a barrier between blood constituents and the arterial wall. Perturbations of this protective function, such as occur following endothelial injury, could lead to increased entry of plasma proteins [18] and lipoproteins [19] into the subendothelium. Our results demonstrate that psychosocial stress causes increased endothelial injury in the area surrounding the intercostal artery orifices. Endothelial damage resulting from hypercholesterolemia and pharmacologically-induced sympathetic activation is similarly focused at hemodynamically vulnerable branched areas [20,12]. This pattern of damage is relevant as it could possibly explain the preferential localization of atherosclerotic plaques to these regions. In a thorough description of the arterial intima, it was concluded that the thickness of it varies along the arterial tree, and local thickening is normal in branched areas [21]. Intimal cushions can be found in such areas in animals that are not normally considered to have an intima [21]. A gentle mechanical endothelial removal from rat carotid arteries led to the formation of a thin, smooth muscle cell rich intima [22]. It is tempting to speculate that endothelial injury in
circumostial areas can elicit trophic response in the intima, and that if this recurrent injurious stimuli occurs in even moderately hypercholesterolemic individuals it may lead to the initiation of an atheromatous plaque. Moreover, individual differences in circumostial endothelial damage could progress over time to differences in atherosclerosis extent.

One minor aim of the current study was to evaluate the effect of psychosocial stress and $\beta$-blockade on the coronary arteries. Unfortunately, while the Häutchen technique was effectively applied to the coronary arteries, a quantitative comparison of injury in circumostial and non-branched areas was not possible because of the irregular size and location of the branching points in the coronary arteries. Despite this difficulty, inspection of these sites suggested the presence of considerable injury in circumostial areas and the relative absence of such injury in non-branched areas. Future studies should be directed at quantitative assessment of the effects of psychosocial stress on endothelial integrity in non-branched and circumostial areas in the coronary arteries.

A second minor aim concerned a comparison of the effect of lipophilicity in inhibiting psychosocial stress-induced endothelial injury. The results indicated that atenolol and metoprolol inhibited stress-induced endothelial injury in the circumostial areas of the descending thoracic aorta to a similar extent. The pooled data from the stressed, $\beta$-blocked groups indicated that the adverse effects of psychosocial stress on the endothelium in circumostial areas of the descending thoracic aorta were sympathetically mediated, via $\beta_1$-adrenoceptor activation. This conclusion is also supported by the observation that $\beta$-blockade with metoprolol prevented injury to endothelial cells in circumostial areas of the descending thoracic aorta in rabbits subjected to a direct sympathetic activation by chloralose anesthesia [12].

Using the method of immunohistochemical detection of injured endothelial cells on Häutchen preparations from monkey coronary arteries gave new insights regarding endothelial integrity and the appearance of these pathobiologically interesting sites. As described, injured endothelial cells were only estimated remote from the areas of the small artery ramifications seen in the coronary arteries, although many injured cells appeared to be present in these circumostial areas. The low frequency of injured endothelial cells found in the non-branched areas of the coronary arteries and the descending thoracic aorta could be due to the fact that the endothelium at these sites is subjected to lower injurious stimuli, or that the endothelium is more resistant to such stimuli than the endothelium at the circumostial sites.

4.1. This study has certain limitations

Capturing and excess handling is stressful for the animals which in turn makes it difficult to obtain reliable basal values of plasma catecholamines and blood pressure. Therefore, to evaluate adrenergic activation, a portable electrocardiogram telemetry transmitter unit was used for frequent sequential heart rate recordings during the $2 \times 24$ h baseline and experimental periods. Despite randomization, the monkeys in the stressed groups had significantly higher baseline heart rate values than the monkeys in the non-stressed groups. This imbalance probably did not influence the experimental outcome as regards endothelial injury in the stressed groups for two reasons: firstly, the possible endothelial injury occurring during the baseline period would be repaired by the time of necropsy, because injured cells only reside in the endothelium for approximately 1–2 days [23]; and secondly, although the highest baseline heart rate values were recorded in the atenolol and metoprolol stressed groups, the endothelial injury data from the circumostial areas of the descending thoracic aorta from these groups did not differ from those obtained from the corresponding non-stressed groups. The experimental heart rate values were adjusted for differences at baseline in the subsequent comparisons of heart rates between the groups.

In conclusion, we observed that psychosocial stress involving the introduction of target monkeys to new social groupings was associated with significantly more injured endothelial cells than in the unstressed controls, as seen in lesion-prone areas around the intercostal artery orifices. At these same sites, the stress effect was inhibited by $\beta$-blockade, which leads us to conclude that psychosocial stress-induced endothelial injury is sympathetically mediated via $\beta_1$-adrenoceptor activation. Together with earlier studies in monkeys [2,9] and an investigation in rabbits demonstrating a similar pattern of endothelial injury in response to pharmacologic activation of the sympathetic nervous system [12], these data supports the idea of psychosocial stress via $\beta_1$-adrenergic activation as a major precipitating and exacerbating factor in atherosclerosis.

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