Gender and Baboon Aortic Steroid Hormone Receptors

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To examine the potential of steroid hormones to serve as putative regulators of aortic cell function, we defined hormone receptor content and distribution in intact baboons. Total androgen receptor content in baboon aortic, thoracic, and abdominal aorta of young mature males was indistinguishable from that of proestrus females. However, 30% to 40% of male aortic androgen receptors were in the nuclear fraction, whereas all aortic androgen receptors of proestrus females were in the cytoplasmic fraction. Cytoplasmic fraction estrogen receptor content of aortic arch and thoracic aorta of Intact males was indistinguishable from that of proestrus females. However, cytoplasmic fraction estrogen receptor content of abdominal aorta of proestrus females was significantly greater than that of males. Nuclear fraction estrogen receptors were not detectable in either male or proestrus female baboon aortas. To assess effects of endogenous estrogen on aortic progesterone receptor content, we quantified cytoplasmic fraction progesterone receptors and found that content of proestrus female aortic arch was not significantly different from that of males. However, cytoplasmic fraction progesterone receptor content of thoracic and abdominal aorta of proestrus females was significantly higher than that of males. To determine whether differences in aortic receptor content or distribution were associated with changes in aortic cell function, we quantified the activity of two enzymes of glycosaminoglycan metabolism. Aortic B-glucuronidase activity was not different in male or proestrus female baboons. Uridine diphosphate glucose (UDPG) dehydrogenase activity of aortic arch and thoracic aorta of proestrus female baboons was not different from that of males; however, UDPG dehydrogenase activity of abdominal aorta of proestrus females was greater than that of males. Our studies establish endogenous androgen regulation of intracellular distribution of baboon aortic androgen receptors and imply that these receptors are physiologically functional. The higher cytoplasmic fraction progesterone receptor content of female thoracic and abdominal aorta, as compared to that of males, suggests aortic estrogen receptors also are physiologically functional. These findings establish that baboon aortic steroid hormone receptor content and distribution is sexually dimorphic, as is the case for rodents. However, the essential identity of B-glucuronidase or UDPG dehydrogenase activity in male and female baboon aortas indicates that baboon aortic glycosaminoglycan metabolism is not sexually dimorphic. The fact that aspects of aortic steroid hormone receptor homeostasis and steroid hormone regulation of aortic UDPG dehydrogenase and B-glucuronidase activity significantly differ in baboons and rodents implies complex effects of steroid hormones which may be species-specific. (Arteriosclerosis 7:248–255, May/June 1987)

It is well established that men have a higher incidence of coronary heart disease (CHD) than age-matched females; however, the underlying mechanism is not well understood. Epidemiological studies also have established a higher incidence of CHD in postmenopausal women compared with premenopausal individuals. Moreover, the total mortality of postmenopausal estrogen-only users is decreased compared with nonusers. Recent studies demonstrated that ovarietomy of diet-induced hypercholesterolemic cynomolgus macaques increased atherosclerosis two- to tenfold in coronary, carotid, and iliofemoral arteries and aorta when compared to controls. In addition, the extent of coronary atherosclerosis in diet-induced hypercholesterolemic, intact female cynomolgus macaques with ovarian dysfunction was similar to that of diet-induced hypercholesterolemic ovariectomized females. These studies suggest a role for estrogen in CHD. There is a sex difference in selected enzyme activities involved in rabbit and rodent aortic glycosaminoglycan and cholesterol metabolism. A sex difference was also observed in the contractile response of isolated rabbit and rat aortas or canine coronary arteries to prostaglandin analogues. These differences may be mediated by steroid hormones as demonstrated by experiments performed in gonadectomized and exogenous steroid hormone-treated animals. In vitro studies demonstrated that collagen production by cultured bovine aortic smooth muscle cells is diminished by estrogen and that estrogen decreases proliferation of cultured rabbit aortic smooth
muscle cells. Estrogen increases and testosterone decreases prostacyclin production by cultured rat aortic smooth muscle cells.

 Autoradiographic and biochemical data established androgen binding components in rat, canine, and baboon cardiovascular. Similar studies established estrogen binding components in rat, rabbit, and baboon cardiovascular. Using biochemical methods, we showed that rat aortic androgen and estrogen receptor content is sex-invariant; however, distribution is sexually dimorphic. We further established that rat aortic estrogen receptors are physiologically functional by demonstrating estrogen enhancement of aortic progesterone receptor content.

To study the applicability of findings in rodent aortas to a higher mammalian species, we examined the effects of endogenous hormones on baboon aortic steroid hormone receptor content and distribution and selected enzymes of glycosaminoglycan metabolism. In this report, we present the results of these studies.

Methods

Animals

Young mature male and female baboons (Papio sp.) were obtained from the colony at Southwest Foundation for Biomedical Research. These animals were 7 to 8 years old and had not been subject to prior experimentation. All females had three consecutive, consistent cycles prior to sacrifice at the time of initial maximum sex skin turgescence.

Chemicals

The following chemicals: [17α-methyl-3H]R1881 (methyltrienolone, specific activity 87 Ci/mmol), [1,2,4,5,6,7,16,17-3H]5α-dihydrotestosterone (5α-DHT, specific activity 208 Ci/mmol), [11β-methoxy-3H3]R2858 (moxestrol, specific activity 79.2 Ci/mmol), [2,4,6,7,16,17-3H6(A/)]estradiol (specific activity 134 Ci/mmol), [14C(U)]uridine diphosphate glucose (UDPG, specific activity 343 mCi/mmol), and radioinert steroids not enumerated above were obtained from Steraloids, Incorporated, Wilton, New Hampshire. Human γ-globulin was from Calbiochem-Behring Corporation, La Jolla, California. Dextran T70 was from Pharmacia Fine Chemicals, Incorporated, Piscataway, New Jersey. Hydroxylapatite (DNA grade, Bio-Gel HTP) was from Bio-Rad Laboratories, Richmond, California. Other materials were the highest quality reagent grade available from the manufacturer. All solutions were prepared in water that had been distilled, deionized, and redistilled from glass.

Preparation of Cytoplasmic Extracts and Quantification of Total Cytoplasmic Androgen, Estrogen or Progesterone Receptors

Baboons were restrained, sedated with an intramuscular injection of ketamine (15 mg/kg), and exsanguinated. The aorta, beginning at 0.5 cm above the left ventricle and ending at the iliac bifurcation, was divided into the aortic arch, thoracic aorta, and abdominal aorta at the ligamentum arteriosus and celiac artery. Tissues were wrapped in aluminum foil, sealed in heat-sealed packets, frozen by compression between blocks of dry ice, and transferred without delay to a -90°C freezer where they were stored until analyzed. All procedures were performed in accordance with guidelines established by the Department of Health and Human Services for the treatment of laboratory animals and were approved by the Animal Research Committee of the Southwest Foundation for Biomedical Research. Aortic cytoplasmic extracts were prepared using a slight modification of previously described methods. In brief, frozen tissues were thawed on ice and subsequent procedures were performed at 2° to 4°C. Tissues were finely minced and then homogenized in a glass-glass homogenizer in five to four volumes of buffer TEDS (50 mM Tris-HCl, 0.1 mM EDTA, 10 mM dithiothreitol, 380 mM sucrose, pH 7.4). Homogenates were centrifuged at 50,000 g at 2°C for 5 minutes, and supernatants were made 20 mM in sodium molybdate, 2.2 mM in phenylmethylsulfonyl fluoride (PMSF), and incubated at 2°C with 0.1 volume of dextran γ-globulin coated charcoal (DGCC) suspension (5% charcoal, 0.5% dextran, 1% γ-globulin, wt/vol) in buffer TED — buffer TEDS without sucrose — for 15 minutes at 200,000 × g. Nonspecific binding was determined in each receptor assay by incubating a parallel series of identical samples that contained radiolabeled ligand and 100-fold molar excess of cortisol to block ORG 2058 binding to corticosterone receptor. These were incubated at 2°C for 20 to 24 hours. Incubation mixtures for androgen receptor quantification contained radiolabeled R1881 (0.1 to 5 nM) and 100-fold molar excess of triamcinolone acetonide to block R1881 binding to progesterone receptors. These were incubated at 2°C for 20 to 24 hours. Incubation mixtures for estrogen receptor quantification contained radiolabeled R2858 (0.25 to 5 nM). These were incubated at 30°C for 4 hours. Nonspecific binding was determined in each receptor assay by incubating a parallel series of identical samples that contained radiolabeled ligand and 100-fold molar excess of the corresponding radiolabeled ligand. Separation of bound and free radioligand was by either the DGCC (androgen and progesterone receptors) or hydroxylation profile (estrogen receptors) method described previously. The specific binding data were evaluated by the method of Scatchard and as double reciprocal plots. The receptor content of cytoplasmic and nuclear fractions was determined by full range saturation analyses. Two classes of aortic progesterone binding components were detected.
man. The second class of progesterone binding components was similar to type II estrogen receptors and was of lower affinity and higher capacity.

Preparation of Nuclear Extracts and Quantification of Total Nuclear Androgen or Estrogen Receptors

Aortic nuclear extracts were prepared exactly as previously described. Nuclear androgen receptors were quantified by incubation at 2°C for 18 to 24 hours, whereas nuclear estrogen receptors were quantified by incubation at 30°C for 4 hours. Incubations contained, in a final volume of 440 μl buffer BBPP (20 mM sodium barbital, 1.5 mM EDTA, 150 mM KCl, 5 mM diethiothreitol, 5 mM piperidoxal-5'-phosphate, 20% glycerol, vol/vol, pH 8.0, at 20°C), 200 μl nuclear extract and various concentrations, 0.5 to 5 nM, of radiolabeled 5α-DHT (androgen receptors) or estradiol-17β (estrogen receptors). Nonspecific binding was determined in a parallel series of identical incubations that additionally contained 100-fold molar excess radioinert ligand. Specific binding was calculated as described in the preceding section.

Enzyme Assays

Aortic homogenate 50,000 g supernatant, devoid of sodium molybdate and PMSF, was obtained during preparation of cytoplasmic extracts for steroid hormone receptor determinations and was used to quantify β-glucuronidase or uridine diphoshate glucose (UDPG) dehydrogenase activity. Tissue extract was stored at -90°C until used for these assays. β-Glucuronidase activity was measured by a modification of the method of Schrecker and Chirigos. The complete assay mixture contained tissue extract diluted four-fold with sodium acetate (pH 4.5) and 1 mM phenolphthalein-β-d-glucuronic in a total volume of 250 μl 0.2 M sodium acetate (pH 4.5). After incubation at 37°C for 1 hour, 0.5 μl of solution containing 0.5 M glycine, 0.2% sodium dodecyl sulfate (SDS) (pH 10.45) was added. Blanks contained tissue extract included after addition of the glycine/SDS solution. After centrifugation at 2,000 g for 5 minutes, the product was quantified by measuring 550 nm absorbance. One international unit (IU) of enzyme activity produced 1 μmol phenolphthalein per minute at 37°C. The activity of UDPG dehydrogenase was measured by a modification of the method of Felsher et al. The complete assay mixture contained tissue extract, 0.75 mM UDP-[U-14C]-glucose and 3.0 mM NAD in a total volume of 60 μl 0.1 M glycine (pH 8.7). After incubation at 30°C for 1 hour, the nucleotide sugars were enzymatically hydrolyzed and the product, glucuronic acid, was separated from glucose by thin-layer chromatography on DEAE-cellulose-coated plastic sheets using ethanol/water (1:1) as solvent. Strips were dried, divided into 1- to 2-cm sections, and the radioisotope was quantified by scintillation spectrometry. The product, glucuronic acid, which remained near the origin, was well separated from the glucose, which traveled with the solvent front. One unit of enzyme activity produced 1 nmol uridine 5'-diphosphoglucuronic acid per minute.

Other Methods

Protein was determined by the procedure of Lowry et al. by the use of bovine serum albumin (BSA) as the standard. DNA was determined by the fluorometric method of Vytasek using salmon testes DNA as the standard. The radioimmunoassay to quantify plasma testosterone and estradiol content was performed by use of antibody prepared against the 19-O-carboxymethyl ether derivative of testosterone conjugated to BSA or estradiol-6-O-carboxymethyl-oxyime bovine thyroglobulin, respectively. Antibody to estradiol was provided by Delwood C. Collins. Radioisotope was quantified in either a Beckman 7800 or 7500 scintillation spectrometer and most samples were counted to 2% precision (at the 95% confidence level). Either the paired t test or multiway analysis of variance was used to assess significance of observed differences.

Results

Relation between Sex and Baboon Aortic Androgen Receptor Content and Distribution

Because the physical and kinetic properties of cytoplasmic androgen receptors of baboon aorta and myocardium are similar and the tissue mass of myocardium is manyfold more abundant than that of aorta, we used baboon myocardium to validate an exchange assay (Figure 1) for measurement of total cytoplasmic androgen receptors. We established that replacement of radiolabeled R1881 bound to baboon myocardium androgen receptors by radioinert R1881 occurred with a 1/2 of approximately 4 hours and that exchange was essentially complete after 20 hours.

Figure 1. Effect of incubation interval and temperature on displacement of R1881 bound to baboon myocardial cytoplasmic androgen receptors. Incubation mixture initially contained 10 nM radiolabeled R1881 (○). After an initial 2-hour incubation at 2°C, radioinert R1881 was added to 1 μM (●). Subsequent incubations were performed at 15°C (A) or 2°C (B). Separation of bound and free steroid by DGCC treatment was performed at 2°C.
to 24 hours of incubation at 2°C (Figure 1). Ligand exchange at 2°C occurred in the absence of receptor inactivation, whereas significant loss of receptor binding occurred during incubation at 15°C. Consequently, we used incubation at 2°C to quantify total androgen receptor content and to determine the effect of endogenous androgen on aortic androgen receptor content and distribution in intact young mature male and proestrus female baboons.

Representative saturation analyses for quantification of baboon aortic cytoplasmic and nuclear androgen receptors (Figure 2) established that our protocols measure limited capacity, high affinity binding components. The properties of these binding species (data not shown) were identical to those previously established for aortic androgen receptors. Total androgen receptor content of aortic arch, thoracic and abdominal aorta were sex-independent; however, distribution was sexually dimorphic (Table 1). All aortic receptors of proestrus female baboons were localized in the cytoplasmic fraction, and the nuclear androgen receptors could not be detected, whereas in males, 30% to 40% of aortic androgen receptors were localized in the nuclear fraction. The dissociation constant for cytoplasmic and nuclear fraction androgen receptors in different aortic segments of male and proestrus female baboons was similar. The mean dissociation constant for cytoplasmic and nuclear fraction androgen receptors, respectively, was 0.10 ± 0.02 (n = 30) and 2.12 ± 1.62 nM (n = 15).

**Relation between Sex and Baboon Aortic Estrogen and Progesterone Receptor Content and Distribution**

To evaluate the effect of endogenous estrogen on estrogen receptor content and distribution and cytoplasmic progesterone receptor content, we quantified total cytoplasmic and nuclear estrogen receptor and cytoplasmic progesterone receptor content in intact young mature male and proestrus female baboon aortas. The nuclear fraction estrogen receptors could not be detected in either male or proestrus female baboon aorta. Three of five males and one of five proestrus females did not have detectable aortic arch cytoplasmic fraction estrogen receptors (Table 2).

![Figure 2](http://atvb.ahajournals.org/)

**Figure 2.** Typical saturation data for quantification of androgen receptors in baboon aortic cytoplasmic (upper panels) and nuclear extracts (lower panels). Preparations were incubated with various concentrations of radiolabeled ligand and binding was quantified as described. **Panels A.** Observed saturation data; ○, total binding; ▲, nonspecific binding; ●, specific binding. **Panels B.** Scatchard plot of the data in Panels A.
The mean cytoplasmic fraction estrogen receptor content of male baboon aortic arch and thoracic aorta was not significantly different (p > 0.05) from that of proestrus females. However, the cytoplasmic fraction estrogen receptor content of proestrus female baboon abdominal aorta was significantly higher than that of males, p < 0.05. The dissociation constant for cytoplasmic fraction estrogen receptor in different segments of male and proestrus female aortas was similar. The mean dissociation constant for all analyses was 0.62 ± 0.36 nM (n = 26). The characteristics of the saturation data and the properties of the estrogen binding components quantified in the present studies were identical to those previously described for rodent and baboon aortic estrogen receptors.

Two classes of cytoplasmic fraction progesterone binding components were detected in all preparations from male or female aortas. Low affinity, type I, cytoplasmic progesterone binding component content ranged from 100 to greater than 5000 fmol/mg DNA. No specific pattern of low affinity binding component content was observed in different segments of the aorta. Cytoplasmic progesterone receptor content (high affinity, type I binding components) of proestrus female baboon aortic arch was not significantly different from that of males (p > 0.05). Cytoplasmic progesterone receptor content of proestrus female thoracic and abdominal aortas was 40% and 60%, respectively, higher than that of males. The sex differences were significant (p < 0.05). The dissociation constants for high and low affinity cytoplasmic fraction progesterone binding components were similar in different aortic segments. The mean dissociation constant for all analyses was 7.22 ± 2.69 nM (n = 21) for type II binding component.

The properties of progesterone receptors quantified in the present studies were identical to those previously described for rodent and baboon aortic progesterone receptors.

### Aortic Enzyme Activities

To evaluate the effect of endogenous steroid hormone on enzymes involved in aortic glycosaminoglycan metabolism, we measured UDPG dehydrogenase and 8-glucuronidase activities in male and proestrus female baboon aortas under conditions of limiting enzyme. No significant difference characterized UDPG dehydrogenase activity of aortic arch and thoracic aorta of male and female baboons. However, UDPG dehydrogenase activity of female baboon abdominal aorta was significantly higher than that of males (p > 0.05) (Table 3). We did not find a sex difference in 8-glucuronidase activity of baboon aorta (p > 0.05). The mean 8-glucuronidase activity of male and female baboon aorta was 11.7 ± 4.5 (mean ± SD, n = 33) mIU/g tissue.

### Discussion

The steroid hormone receptor content of cardiovascular tissue is low. Meaningful quantification of these receptors requires a highly reproducible and sensitive assay method. In previous studies, we established that intraassay coefficients of variation for quantification of baboon myocardial cytoplasmic fraction estrogen and progesterone receptors were 9.2% and 8.8%, respectively. The mean interassay coefficients of variation for quantification of baboon aortic cytoplasmic androgen and progesterone receptor content in the present study were 21% and 20%, respectively (Tables 1 and 2), and are

### Table 1. Cytoplasmic and Nuclear Androgen Receptor Content of Baboon Aortas

<table>
<thead>
<tr>
<th>Sex</th>
<th>Aortic arch</th>
<th>Thoracic aorta</th>
<th>Abdominal aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>N</td>
<td>C</td>
</tr>
<tr>
<td>Male</td>
<td>164 ± 23</td>
<td>76 ± 21*</td>
<td>169 ± 24*</td>
</tr>
<tr>
<td>Female</td>
<td>224 ± 57</td>
<td>0</td>
<td>278 ± 56</td>
</tr>
</tbody>
</table>

Data are the mean ± SD, n = 5. Values were obtained by linear regression analysis of double reciprocal plots of the saturation data. Mean male and female plasma testosterone contents (ng/dl), respectively, were 569 ± 346 (mean ± SD) and 250 ± 208. The average DNA content of baboon aorta is 2.34 rng/g tissue. C, cytoplasmic; N, nuclear.

*Significantly different from the paired female value; p < 0.05, paired t test.

### Table 2. Cytoplasmic Fraction Estrogen and Progesterone Receptor Content of Male and Female Baboon Aortas

<table>
<thead>
<tr>
<th>Sex</th>
<th>Aortic arch</th>
<th>Thoracic aorta</th>
<th>Abdominal aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ER</td>
<td>PR</td>
<td>ER</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11 ± 14*</td>
<td>506 ± 123</td>
<td>30 ± 6</td>
</tr>
<tr>
<td>Female</td>
<td>20 ± 14*</td>
<td>833 ± 155</td>
<td>38 ± 13</td>
</tr>
</tbody>
</table>

Data are mean ± SD, n = 5. Values for ER were obtained by linear regression analysis of double reciprocal plots of the saturation data. Values for PR were obtained after applying the correction protocols of Rosenthal and Fieldman to saturation data plotted according to the method of Scatchard. Tissues were from the same baboons described in Table 1. ER, estrogen receptor; PR, progesterone receptor.

*Three of five males and one of five females did not have detectable cytoplasmic fraction estrogen receptors.
†Significantly different from the paired female value; p < 0.05, paired t test.
Table 3. UDPG Dehydrogenase Activity in Male and Female Baboon Aortas

<table>
<thead>
<tr>
<th>Sex</th>
<th>Enzyme activity (units/g tissue)*</th>
<th>Aortic arch</th>
<th>Thoracic aorta</th>
<th>Abdominal aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>117.0 ± 18.1</td>
<td>124.0 ± 29.6</td>
<td>91.7 ± 9.8†</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>115.4 ± 16.0</td>
<td>143.4 ± 22.6</td>
<td>147.3 ± 31.8</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean ± SD, n = 5.
*One unit of enzyme activity produces 1 μmol phenolphthalein per minute at 37°C.
†Significantly different from females; p < 0.05, paired t test.

The results of these studies suggest that long-term ovariotomy may alter baboon aortic progesterone binding components. Comparable levels of androgen, estrogen, and progesterone receptors are found in rat aortas. In contrast, canine aortic estrogen receptor content is higher than that of androgen receptors and a low level of progesterone receptor is demonstrable only in aortas of pregnant or estrogenized female dogs. We find that baboon aortic progesterone receptor content is higher than that of androgen receptor and both greatly exceed baboon aortic estrogen receptor content (Tables 1 and 2). These data suggest that baboon aortic estrogen receptors do not appear to decrease the significance of potential effects of estrogen on aortic cell function, because aortic progesterone receptor content is significantly elevated in proestrus female baboon aorta when compared to that of males.

In ovariotomized female baboons, the estrogen receptor content was highest in the abdominal aorta and lowest in the aortic arch. In contrast, cytoplasmic fraction progesterone receptor content was highest in the aortic arch and lowest in the abdominal aorta. A similar regional heterogeneity for cytoplasmic estrogen and progesterone receptor distribution was observed for both intact male and female baboon aortas (Table 2). Consequently, regional heterogeneity of baboon aortic steroid hormone receptors appears to be independent of sex.

Aortic β-glucuronidase activity was not different in male and female baboons. UDPG dehydrogenase activity of aortic arch and thoracic aorta of proestrus female baboons also was indistinguishable from that of males (Table 3). However, UDPG dehydrogenase activity of abdominal aorta of proestrus females was greater than that of males. These findings imply that baboon aortic glycosaminoglycan metabolism principally is not sexually dimorphic. Ovariotomy of male rabbits decreases aortic UDPG dehydrogenase activity and increases aortic β-glucuronidase activity. In contrast, ovariotomy of female rats increases aortic UDPG dehydrogenase activity and decreases aortic β-glucuronidase activity. Effects of gonadectomy are reversed by testosterone treatment of male or estrogen treatment of female rodents. Moreover, β-glucuronidase activity of male mouse aorta is greater than that of females. The sex difference is abolished by orchietomy of male mice or testosterone treatment of female mice. The contradictory nature of the results of these enzyme studies among rabbits, rats, mice, and baboons is notable and may, in part, reflect species differences.

Our current and prior studies to characterize cardiovascular steroid hormone receptors and their regulation by sex hormones were restricted to rodent and baboon aortas. Choice of aorta for these evaluations principally reflected limitations of tissue availability. Although sex differences in fatty streak formation characterize prepubertal human male and female aortas, adult differences in cardiovascular disease principally reflect sexual dimorphism in
the incidence and severity of coronary artery plaque formation. Because morphologic and physical properties of the aorta and coronary arteries differ significantly (reviewed in reference 1), extrapolation of our findings in aorta to the coronary arteries may be inappropriate. However, the fact that we demonstrate potential for steroid hormones to regulate aspects of aortic cell function establishes precedence for potential steroid hormone regulation of coronary artery cell function. Only direct analysis will determine if steroid hormones regulate coronary artery cell function and whether such effects are sexually dimorphic.

Acknowledgments

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