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Short communication

Gender difference in levels of α_2-adrenoceptor mRNA in the rat tail artery

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Abstract

To investigate the hypothesis that differing mRNA levels underlie gender differences in the contractile response of the rat tail artery, α_2-adrenoceptor mRNA was measured using in situ hybridization. Messenger RNA for the α_{2A}- and α_{2C}-adrenoceptor subtypes was found localized to the smooth muscle layer. There was no detectable mRNA present for the α_{2B}-adrenoceptor subtype. Levels of α_{2C}-adrenoceptor mRNA were greater in female compared to male tail arteries (417 ± 35 vs. 263 ± 38 dpm/mg, $P = 0.01$), while levels of α_{2A}-adrenoceptor mRNA were the same in both sexes. Levels of α_2-adrenoceptor mRNA may parallel levels of functioning protein present in the rat tail artery.

Keywords: α_2-Adrenoceptor; Tail artery rat; mRNA

1. Introduction

α_2-Adrenoceptor mRNA levels have been shown to be altered by sex hormones in various cells, including those of the kidney and adipocytes (Gong et al., 1995; Pecquery et al., 1995). Others have shown that the effects of the α_2 agonist clonidine on current through Ca^{2+} channels in rat tail artery vascular smooth muscle cells is greater in females compared to males (Song et al., 1996). In the rat tail artery, we have found that the contractile response to α_2-adrenoceptor activation varies with sex such that responses are greater in arteries from males compared to females (Chen, D.C., Krause, D.N., and Duckles, S.P., unpublished observations). However, the subtype(s) of α_2-adrenoceptor present in rat tail artery smooth muscle and whether levels of receptor protein vary with sex are as yet unknown.

Therefore, the purpose of this study was to determine whether expression of α_2-adrenoceptors varies with sex in the rat tail artery. Because currently available α_2 agonists and antagonists are not subtype-specific, molecular methods are necessary in order to determine the subtypes of α_2-adrenoceptor present in a complex tissue. To this end, levels of mRNA for the three α_2-adrenoceptor subtypes (α_{2A}, α_{2B} and α_{2C}) were measured in the rat tail artery using in situ hybridization, and arteries from male and female animals were compared.

2. Materials and methods

Three month old male and non-cycled female Fischer-344 rats, weighing 232 ± 4 g and 141 ± 3 g respectively, were obtained from the National Institutes of Health-National Institute on Aging (NIH-NIA) colony. Animals were kept for a minimum of two weeks before study in a room maintained at 24°C with a 12 h light–dark cycle and unlimited access to both food and water.

2.1. Tissue preparation

Each rat was decapitated, and the tail artery or brain removed and rapidly frozen in −30°C isopentane. Tail arteries were cut into approximately 30 mm sections and mounted in TissueTek. Tissues were stored at −70°C for future use. Rat tail artery sections 8 μm thick and brain sections 20 μm thick were cryostat-cut, mounted onto poly-L-lysine coated slides and fixed with 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS), pH 7.4

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at room temperature, for 1 h. Sections were then washed in PBS, air-dried, and stored, desiccated, at −20°C for in situ hybridization. Successive sections from each tissue were cut for use with each of the antisense and sense riboprobes for each of the three subtypes of the α_2-adrenoceptor. Brain tissue was used as a positive control.

2.2. Probe preparation

A 3.0 kb cDNA encoding the α_{2A}-adrenoceptor and a 2.8 kb cDNA encoding the α_{2B}-adrenoceptor were obtained from Dr. Kevin Lynch, University of Virginia. [^{35}S]-labeled uridine triphosphate (UTP; Dupont NEN, Boston, MA) was used in synthesizing cRNA riboprobes for in situ hybridization. These probes were further subjected to alkaline hydrolysis to yield products with average sizes of 600 bases. A 4.6 kb cDNA encoding the α_{2C}-adrenoceptor was also obtained from Dr. Lynch. A 242-bp fragment encoding the third intracellular loop of the α_{2C} receptor was used as a template to synthesize ^{35}S-labeled α_{2C} receptor riboprobes (Winzer-Serhan et al., 1997b). Probes synthesized in the antisense direction were used to determine the subtypes of α_2-adrenoceptor mRNA present in the tissue, and those made in the sense direction were used to assess non-specific hybridization.

2.3. In situ hybridization

Tissue sections were processed for in situ hybridization following a modified version of the method described by Simmons et al. (1989). Males and females were always processed together, over 1 or 2 runs of in situ hybridization for each subtype of cRNA probe. Briefly, slide-mounted artery sections were incubated with 20 μg/ml proteinase K, and brain sections, 0.05 μg/ml proteinase K, for 10 min at 22°C, acetylated, dehydrated through graded ethanols, and air-dried. Sections were then incubated for approximately 18 h at 60°C with a hybridization solution containing cRNA probes labeled with [^{35}S]UTP (approximately 1×10^7 c.p.m./ml) in the antisense or sense direction. Sections were then incubated with RNase A (20 μg/ml) for 30 min at 37°C. This was followed by 4×5 min high-stringency washes of decreasing salinity from $2 \times$ standard saline citrate buffer (SSC: 0.37 M NaCl and 0.037 M sodium citrate) to $0.5 \times$ SSC buffer at 22°C, and a final 30 min wash in $0.1 \times$ SSC buffer at 70°C. Tissue sections were dehydrated, dried in a stream of cool air, and apposed to β-max film for 3–7 days at 4°C.

2.4. Data analysis

Autoradiograms were quantified with a computer-based image analysis system (MCID, Imaging Research, St. Catherine, Ontario, Canada) using calibrated standards of reference. A calibration curve of optical density against radioligand concentration (dpm/mg tissue) was used with [^{14}C] brain paste standards of known radioactivity. Optical densities in discrete regions of tail artery radiographic images were measured, and corresponding values of radioactivity were determined by interpolation from the standard curve. Levels of mRNA (specific hybridization) were calculated by subtracting levels of radioactivity in sections exposed to probes made in the sense direction (non-specific hybridization) from levels of radioactivity in sections exposed to probes made in the antisense direction (total hybridization). Calculations of specific hybridization were made by comparison of levels of non-specific and specific hybridization in adjacent tissue sections from the same animal. Because the specific activities of the cRNA probes used in this study were not determined, these concentration measurements do not represent the absolute levels of mRNA in the tissue. Therefore, comparison of mRNA density is valid for groups within each subtype of α_2-adrenoceptor mRNA, but not between α_2-adrenoceptor subtypes.

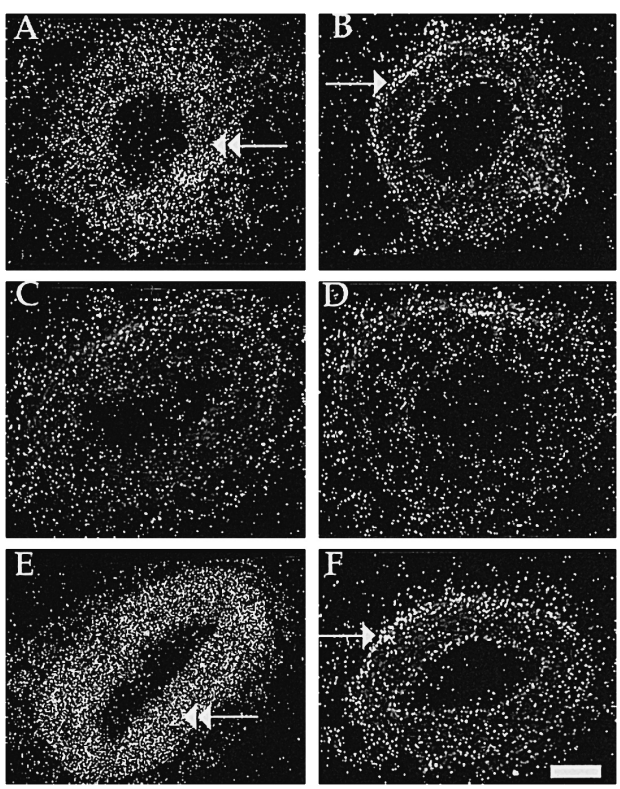

Fig. 1. Representative autoradiograms of rat tail artery labeled for α_{2A} (A, B), α_{2B} (C, D), and α_{2C} (E, F)-adrenoceptor mRNA. Antisense probe labeling (A, C, E) represents total hybridization, while sense probe labeling (B, D, F) represents non-specific hybridization. After probe labeling as described in Section 2, tissue sections were emulsion dipped and stained with Cresyl violet. Arrow: non-specific sense hybridization to adventitial layer. Double arrow: antisense hybridization to smooth muscle layer. Scale bar: 100 μm. (A, B) Antisense and sense probe labeling, respectively, for the α_{2A}-adrenoceptor. (C, D) Antisense and sense probe labeling, respectively, for the α_{2B}-adrenoceptor. (E, F) Antisense and sense probe labeling, respectively, for the α_{2C}-adrenoceptor.

2.5. Histology

Tissue sections were emulsion-dipped and stained with Cresyl violet in order to localize mRNA within the tissue. In some instances alternate sections were stained with hematoxylin and eosin to examine tissue histology. Sections were viewed and photographed by transillumination microscopy.

3. Results

3.1. Probe specificity

Probe specificity was confirmed by examination of the pattern of probe hybridization in rat brain sections. For each subtype of α_2-adrenoceptor, the localization of antisense probe hybridization (specific hybridization) was identical to previously published results (Winzer-Serhan and Leslie, 1997; Winzer-Serhan et al., 1997a,b). Sections incubated with sense riboprobes exhibited non-specific localization with low levels of hybridization, representing background hybridization.

3.2. mRNA localization and histology

The endothelial layer was found to be incomplete by visual inspection of hematoxylin and eosin-stained tail artery sections.

Specific hybridization of probes for α_{2A} and α_{2C}-adrenoceptor mRNA was found throughout the smooth muscle layer (n = 6-7 animals, Fig. 1). No specific hybridization of α_{2B}-adrenoceptor mRNA probes was detectable in the smooth muscle layer ($n = 7$ animals, Fig. 1), although specific hybridization in brain sections was comparable to previous reports.

In the adventitial layer, no specific hybridization of α_2-adrenoceptor probes was found. Non-specific hybridization of the sense probe was evident in the adventitial layer (see Fig. 1B,F).

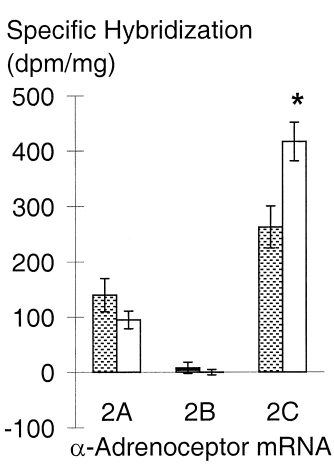

Fig. 2. Density of α_2-adrenoceptor mRNA in the rat tail artery (open bars: females, shaded bars: males, $n = 6–7$ animals). mRNA density (dpm/mg, specific hybridization) was calculated by subtracting levels of radioactivity in sections exposed to probes made in the sense direction (non-specific hybridization) from levels of radioactivity in sections exposed to probes made in the antisense direction (total hybridization). Values are means ± S.E. * Significantly different from male by unpaired *t*-test; $P = 0.01$.

3.3. Effect of gender

The amount of α_{2C} mRNA was 63% higher in female, compared to male, rat tail arteries ($P = 0.01$, $n = 7$ animals, Fig. 2). The amount of α_{2A} mRNA was not significantly different in tail arteries from females, compared to males ($n = 6–7$ animals).

4. Discussion

The most important finding of this study is that there is a sex difference in levels of mRNA for a subtype of α_2-adrenoceptor in vascular smooth muscle tissue. mRNA for α_{2A}-adrenoceptors was found in the smooth muscle layer of tail arteries of both male and female rats. This agrees with the findings of Phillips et al. (1997), who also detected α_{2A} mRNA using reverse transcriptase–polymerase chain reaction (RT-PCR) in the tail artery of tissue pooled from male and female rats. Indeed, mice with a mutant α_{2A}-adrenoceptor lose the peripherally-mediated hypertensive response when α_2 agonists are administered via the femoral artery, but not the carotid artery, suggesting that, depending on the particular vascular compartment involved, α_{2A}-adrenoceptors in the periphery mediate an increase in peripheral resistance (MacMillan et al., 1996).

Although mouse knockout models suggest that the major component of the α_2 adrenoceptor agonist-induced increase in systemic blood pressure is mediated by α_{2B} receptors in peripheral resistance vessels (Link et al., 1996), we did not find any α_{2B} receptor mRNA in the rat tail artery. However, as an artery specialized for thermoregulation, the rat tail artery may not be representative of peripheral resistance vessels in general. In contrast to our findings, Phillips et al. (1997) did detect α_{2B} receptor mRNA by RT-PCR in tail artery homogenate, as well as in homogenates of basilar, mesenteric and pulmonary arteries from Wistar–Kyoto rats. These homogenates consisted of all layers of the blood vessel wall, including the endothelial layer, which was not fully intact in our arterial sections. We used in situ hybridization to localize mRNA to specific layers of the vessel wall without amplification of any mRNA. The previous finding of α_{2B} receptor mRNA may, therefore, be due to contamination of homogenates by other cell types or a strain difference.

In our study, a high density of α_{2C}-adrenoceptor mRNA was present in the vascular smooth muscle layer of the rat tail artery. Similarly, Phillips et al. (1997) 'very strongly detected' α_{2C} mRNA in the rat tail artery using RT-PCR and did not detect or only weakly detected α_{2C} mRNA in the basilar, mesenteric and pulmonary arteries. Mouse knockout models show no effect of loss of the α_{2C}-adrenoceptor on the peripherally-mediated hypertensive response to injection of α_2 agonists (Link et al., 1996), suggesting that the α_{2C}-adrenoceptor is not a major mediator of the systemic cardiovascular effects of α_2-adrenoceptor agonists. This suggests a possible unique role of the α_{2C}-adrenoceptor in the tail artery.

A major role of α_2-adrenoceptors in the rat tail artery is thermoregulation. Injection of an α_2-adrenoceptor antagonist causes an elevation in tail skin temperature in the conscious rat that is not secondary to an increase in core temperature (Redfern et al., 1995). Furthermore, α_{2C}-knockout mice are resistant to the hypothermic (core temperature) effect of α_2-adrenoceptor agonist infusion compared to control mice (Sallinen et al., 1997), indicating some role of the α_{2C} subtype in thermoregulation. Thus, the thermoregulatory role of α_2-adrenoceptors in the tail artery may be mediated by the α_{2C} subtype.

Density of α_{2C}-adrenoceptor mRNA was greater in female, compared to male, tail arteries. The dependence of this effect on gonadal hormone levels, and which hormones might be involved, are subjects of future investigation. Possible sex differences were not addressed in previous studies, which either pooled tissue from male and female animals (Phillips et al., 1997), or only studied male animals (Redfern et al., 1995). Raynaud's disease, characterized by cold-induced digital vasospasm, is five times more common in women than men, and blockade of vasospastic attacks by α_2-adrenoceptor antagonists has been shown (Freedman et al., 1995). Whether or not the pathophysiology of Raynaud's disease involves an aberration of the α_{2C}-adrenoreceptor remains to be investigated.

In conclusion, we have shown that mRNA for both the α_{2A} and α_{2C}-adrenoceptors was present in the smooth muscle layer of the rat tail artery. No α_{2B}-adrenoceptor mRNA was present. Furthermore, the density of α_{2C} receptor mRNA was greater in arteries from females compared to males. Further investigation is needed to determine whether or not the differences in mRNA are reflected at the protein level, but these findings suggest that the function of α_{2C}-adrenoceptors may be enhanced in female arteries. The implications of these findings as they relate to thermoregulation and vasospasm remain to be investigated.

Acknowledgements

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