Androgen Receptor (AR) Immunoreactivity in Rat Pudendal Motoneurons: Implications for Accessory Proteins

Cynthia Jordan

Department of Psychology, University of California, Berkeley, Berkeley, California 94720-1650

Received March 21, 1997; accepted May 3, 1997

Pudendal motoneurons in male rats are located in two sexually dimorphic motoneuronal pools: the spinal nucleus of the bulbocavernous (SNB) and the dorsolateral nucleus (DLN). SNB motoneurons innervate sexually dimorphic muscles bulbocavernous (BC) and levator ani (LA) and the sexually monomorphic external anal sphincter (EAS) muscle. DLN motoneurons innervate either the sexually dimorphic ischiocavernosus (IC) muscle or the sexually monomorphic external urethral sphincter (EUS) muscle. Previous observations indicate that the size of BC, LA, and IC motoneurons in males is sensitive to adult androgen manipulations, whereas the size of EAS and EUS motoneurons is not, raising the question of whether this difference in androgen sensitivity among pudendal motoneurons reflects a difference in androgen receptor (AR) expression. AR immunocytochemistry using the PG-21 antiserum was carried out on spinal cord tissue from normal adult male rats in which specific pudendal motoneuronal subpopulations were identified with retrograde markers. Over 90% of BC, LA, and IC motoneurons displayed AR immunoreactivity in their nuclei. Among motoneurons in the SNB, significantly fewer EAS motoneurons had AR-positive nuclei, which may contribute to the reported failure of EAS motoneurons to morphologically respond to changes in androgen levels. However, within the DLN, despite the fact that IC but not EUS motoneurons are reported to respond to androgen with an increase in soma size, IC and EUS motoneurons had the same proportion of AR-positive nuclei. These results indicate that androgen receptors, while necessary, are not sufficient to confer androgen sensitivity to cells. © 1997 Academic Press

Pudendal motoneurons of the adult male rat are located in two sexually dimorphic motor pools of the lower lumbar spinal cord, the spinal nucleus of the bulbocavernosus (SNB; also referred to as the dorsomedial nucleus or DM) and the dorsal lateral nucleus (DLN, Breedlove and Arnold, 1980; Schroder, 1980; McKenna and Nadelhaft, 1986). The SNB is positioned in the medial aspect of the ventral horn and innervates three perineal muscles in male rats: the bulbocavernous (BC), the levator ani (LA), and the external anal sphincter (EAS). The BC and LA muscles are sexually dimorphic (Wainman and Shipounoff, 1941; Cihak, Gutmann, and Hanzlíková, 1970), present in adult male rats and absent or vestigial in adult females rats, whereas the EAS muscle is present in both sexes. The DLN is positioned in the dorsolateral aspect of the ventral horn and motoneurons in this nucleus innervate either the sexually dimorphic ischiocavernosus (IC) muscle that is present in males and virtually absent in females or the sexually monomorphic external urethral sphincter (EUS) muscle. Reflecting these sex differences in target musculature, the SNB and DLN in adult male rats have about two to three times as many motoneurons as do adult female rats (Breedlove and Arnold, 1981; Jordan, Breedlove, and Arnold, 1982). Whereas both sexes have motoneurons that innervate the sphincter muscles (EAS motoneurons in the SNB and EUS motoneurons in the DLN), adult males have additional SNB and DLN motoneurons that innervate the BC and LA muscles in the SNB and the IC muscle in the DLN.

While SNB and DLN motoneurons, in general, are influenced by gonadal androgens (Breedlove and Arnold, 1981, 1983a, 1983b; Kurz, Sengelaub, and Arnold, 1986; Leedy, Beattie, and Bresnahan, 1987; Matsumoto, Arnold, Zampighi, and Micevych, 1988a; Matsumoto, Micevych, and Arnold, 1988b; Lee, Jordan, and Arnold, 1989; Hodges, Jordan, and Breedlove, 1993), the sexu-
ally dimorphic and sexually monomorphic subpopulations of the SNB and the DLN apparently exhibit different sensitivities to gonadal androgens. For example, whether sexually dimorphic motoneurons of the SNB and DLN survive depends on gonadal androgens in development (Nordeen, Nordeen, Sengelaub, and Arnold, 1985; Sengelaub and Arnold, 1989), whereas the survival of EAS and EUS motoneurons does not, since equal numbers of EAS and EUS motoneurons are present in adult males and females (McKenna and Nadelhaft, 1986) despite the virtual absence of androgens in developing females. Similarly, in adulthood, pudendal motoneurons show different sensitivities to gonadal androgens. The mean size of motoneuronal somata for BC, LA, and IC motoneuronal groups decreases significantly after 4 weeks of castration, whereas the mean size of EAS and EUS motoneurons does not change (Collins, Seymour, and Klugewicz, 1992). Because only the sexually dimorphic pudendal motoneurons respond to gonadal androgens by altering the size of their somata, the question arises as to whether only the androgen-sensitive pudendal motoneurons express androgen receptors (ARs).

The adult SNB and DLN are relatively rich in ARs. For example, data based on steroid autoradiography (Breedlove and Arnold, 1983c) and more recently on AR immunocytochemistry (AR ICC) (Freeman, Padgett, and Breedlove, 1995; Jordan, Padgett, Hershey, Prins, and Arnold, 1997) suggest that most, but not all, adult SNB and DLN motoneurons contain ARs. Thus, EAS and EUS motoneurons may fail to respond to androgen because they lack ARs. The present study set out to determine whether EAS and EUS motoneurons have ARs by combining Fluoro-Gold retrograde labeling with AR ICC. I found that about 70% of EAS and about 90% of EUS motoneurons stain positively for ARs, suggesting that most EAS and EUS motoneurons have ARs, despite the reported insensitivity of their size to adult androgens. These data suggest the possibility that accessory proteins act in collaboration with the AR to mediate the change in soma size induced by androgen.

METHODS

Retrograde labeling. Normal adult male Sprague–Dawley rats obtained from Charles River ranging in body weight (BW) from 316 to 518 g (mean = 420 g) were anesthetized with an ip injection (0.9 ml/kg BW) of ketamine cocktail (containing 100 mg/ml ketamine, 20 mg/ml xylazine, 10 mg/ml acepromazine) and their muscles injected with 5% Fluoro-Gold (Fluorochrome, Inc., Englewood, CO) dissolved in 0.9% NaCl. Except for the EAS, perineal muscles were exposed for Fluoro-Gold injections either via a midline incision through the scrotum (exposing the BC, LA, and IC muscles) or via a midline incision in the skin just rostral to the base of the penis in the peritoneum (exposing the EUS). The EAS was injected through the perianal skin without an incision. Each muscle received multiple (three to five) injections of Fluoro-Gold with the total volume received by any one muscle being 5–10 μl. Prior to closure of the incision with wound clips and/or gut sutures, the injected muscle and the surrounding area were thoroughly rinsed with mammalian Ringer’s to remove excess Fluoro-Gold that may have leaked from an injection site. After 7 or 8 days, animals were sacrificed and their tissues processed for AR ICC.

Controls for nonspecific spread of Fluoro-Gold. Because the LA, BC, and IC all attach to the base of the phallus and are adjacent to one another, there were concerns about nonspecific spread of Fluoro-Gold between these muscles. Thus, the following injection strategy was chosen. For each animal, Fluoro-Gold was injected into one target muscle for SNB motoneurons (BC, LA, or EAS) on one side and into one target muscle for DLN motoneurons (IC or EUS) on the other side. If labeled motoneurons were found in both the SNB and the DLN on one side or were found bilaterally in either nucleus, this would indicate spread of the Fluoro-Gold from the injected muscle to neighboring muscles. In this case, the tissue would be excluded from the quantitative analysis.

Because spread of Fluoro-Gold among targets on the same side for either SNB or DLN motoneurons would not be detected by this strategy, seven experiments were done in which two different retrograde fluorescent labels were used. Animals received unilateral injections of 5–8% Fluoro-Ruby dissolved in NaCl (a 10,000 MW dextran, tetramethylrhodamine, lysine fixable, Molecular Probes, Eugene, OR) into one SNB target (e.g., right BC) and Fluoro-Gold into another (e.g., right LA), as well as injections of Fluoro-Ruby and Fluoro-Gold into the two DLN targets (e.g., left IC and EUS) contralateral to the injected SNB targets (i.e., four muscles injected per animal). Evidence of double-labeled cells in the SNB or DLN would reveal unilateral spread among SNB (or DLN) targets. All other procedures were the same, including multiple small injections into each target, with the total volume received by any one target being 3–10 μl. Since Fluoro-Ruby has a slower transport rate than Fluoro-Gold, with optimal retrograde labeling achieved after 3 weeks (Piehl, Arvidsson, and Cull-
AR Immunoreactivity in Motoneurons

At the time of sacrifice, animals were overdosed with an ip injection of pentobarbital and perfused through the heart with physiological saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The lumbar spinal cord was dissected and postfixed in the same fixative for 2–3 hr at room temperature and then transferred to 20% sucrose in 0.1 M phosphate buffer and refrigerated overnight. The right side of the spinal cords was marked by a longitudinal slit through the right dorsal white matter, and the spinal cords were sectioned transversely at 50 μm on a freezing microtome. Floating tissue sections were rinsed several times in a solution containing 0.1 M phosphate buffer (pH 7.4), 0.1% gelatin, and 0.3% Triton X-100 (PGT) and subjected to an AR ICC protocol detailed in Freeman et al. (1995). In brief, tissue was blocked for nonspecific staining in a solution of PGT containing 10% normal goat serum and then transferred to PGT containing 0.71–1.056 μg/ml of PG-21 antiserum (courtesy of Gail Prins). One-third of the sectioned tissue was incubated in PGT that lacked primary antiserum. After 72 hr at 4°C, tissue was reacted at room temperature using Vectastain ABC Elite kit reagents and nickel-enhanced diaminobenzidine as the chromogen. Tissue was mounted on gel-subbed slides and allowed to dry for at least 24 hr before coverslipping. Mounted tissue was rinsed, dehydrated, cleared in Hemo-de (Fisher Scientific), and coverslipped using DPX (Fluka). Tissue was stored at 4°C in the dark until analysis.

The specificity of the PG-21 antiserum and the reliability of the AR ICC method for detecting ARs in pudendal motoneurons are well established (Freeman et al., 1995; Matsumoto, Arai, and Prins, 1996; Jordan et al., 1997). In brief, preabsorption experiments demonstrate that the PG-21 antiserum binds to a peptide sequence in spinal cord tissue that can be blocked by prior exposure to the immunizing peptide but not to a peptide sequence further along in the AR. In addition, estimates of the number of SNB and DLN motoneurons that contain AR based on PG-21 AR ICC agree well with previous estimates based on steroid autoradiography (Breedlove and Arnold, 1983; Jordan, Breedlove, and Arnold, 1991).

Microscopic and statistical analyses. Control (no primary) tissue was previewed to assess the quality of Fluoro-Gold labeling. If appreciable numbers of SNB and/or DLN motoneurons were labeled, and labeling was on the correct sides without evidence of nonspecific spread, then the tissue exposed to the primary antiserum was quantitatively assessed. An additional criterion also had to be satisfied for Fluoro-Gold labeling in the DLN. Since IC and EUS motoneurons occupy predominantly medial and lateral positions, respectively, in the DLN (Schröder, 1980; McKenna and Nadelhaft, 1986; Collins et al., 1992), injections in the IC were required to yield Fluoro-Gold-labeled cells in the medial aspect of the DLN whereas injections in the EUS were required to yield Fluoro-Gold labeling in the lateral aspect of the DLN.

Under UV illumination, the number of Fluoro-Gold-labeled motoneurons in the SNB and DLN was counted at 200× (Figs. 1a, 1b, 1c, 2c, and 2d). Transmitted light illumination was then used at the same magnification to determine whether the nucleus of each Fluoro-Gold-labeled motoneuron contained AR immunoreactivity (Figs. 1d, 1e, 1f, 2e, and 2f). The density of AR immunostaining was also qualitatively assessed by categorizing the staining in each nucleus as none, light (just detectable), medium, or dark (Fig. 2e shows the range of staining densities). The percentage of Fluoro-Gold-labeled cells with AR-immunopositive nuclei was derived from these counts.

An analysis of variance was used to determine whether AR labeling varied significantly among the various pudendal motoneuronal subpopulations. Separate one-way analyses of variance (ANOVA) were run for the SNB and the DLN (Woodward, Bonett, and Brecht, 1990). For the SNB, a one-way, three-level (corresponding to BC, LA, and EAS motoneuronal subpopulations) analysis was performed and for the DLN, a one-way, two-level (corresponding to IC and EUS subpopulations) analysis was performed. If significant main effects were obtained, specific post hoc comparisons were made. Data are presented as the mean ± standard error of the mean (SEM).

RESULTS

Specificity and robustness of Fluoro-Gold labeling. Fluoro-Gold injections in perineal muscles consistently yielded brightly fluorescing cells in the SNB and DLN 7–8 days later. Fluoro-Gold-labeled cells were also consistently found only ipsilateral to the injection in the expected motor pool. No spinal cords were excluded because of aberrant labeling. Moreover, when Fluoro-Gold and Fluoro-Ruby were injected in different targets of the SNB and DLN, labeled cells were in their expected positions in the spinal cord and there was only 1 doubled-labeled cell of 373 fluorescent cells, demon-
FIG. 1. Representative photomicrographs of AR-immunoreactive spinal cord sections showing Fluoro-Gold retrograde labeling of pudendal motoneurons in the SNB. Epifluorescent UV illumination reveals Fluoro-Gold labeling of levator ani (LA) (a), bulbocavernosus (BC) (b), and external anal sphincter (EAS) (c) motoneurons (arrows) while illumination with transmitted light reveals AR immunostaining in the nuclei of these same Fluoro-Gold-labeled LA (d), BC (e), and EAS (f) motoneurons (arrows). Bar, 100 μm.
stratifying the high degree of specificity of these two labels [based on experiments which yielded >10 Fluoro-Gold- and >10 Fluoro-Ruby-filled cells in either the SNB (n = 2) or the DLN (n = 6)]. Because Fluoro-Gold is a remarkably stable fluorescent label, surviving extensive tissue processing and showing very little fading during the analysis phase, Fluoro-Gold-labeled cells could be easily recognized and counted and their AR-immunostaining characteristics assessed (Figs. 1 and 2). At the completion of the analysis, Fluoro-Gold labeling was still bright and would allow for a subsequent quantitative analysis.

Of the 28 animals used in this study, tissue from 5 animals was excluded because AR immunostaining was poor (n = 2), the caudal extent of the SNB and DLN was damaged (n = 2), or there were too few Fluoro-Gold-labeled cells (n = 1); this left 23 animals from which data were obtained. The EAS muscle proved to be most difficult to label with Fluoro-Gold. To increase the chances of injecting the EAS, 4 of the 8 animals that contributed data to the EAS group received bilateral Fluoro-Gold injections. The mean (± SEM) percentage of AR-positive EAS motoneurons was no different for animals that received unilateral (65 ± 13.00) versus bilateral (73 ± 9.96) injections of Fluoro-Gold.

Occasionally, Fluoro-Gold-labeled cells were found midway in the ventral horn between the SNB and the DLN. This labeling may not, however, reflect nonspecific spread, since their position is consistent with the migratory path of SNB motoneurons in development (Sengelaub and Arnold, 1986). Fluoro-Gold-labeled motoneurons in this intermediate position were not included in the quantitative analysis.

**AR immunostaining for different pudendal motoneuronal subpopulations.** The percentage of motoneurons with AR-immunopositive nuclei varied significantly among pudendal subpopulations in the SNB (main effect: P < 0.002); 95% of both LA and BC motoneurons had AR-immunopositive nuclei, and 90% had medium or dark staining (Figs. 3a and 3b). In contrast, only 69% of EAS motoneurons were AR-immunopositive (Fig. 3a), significantly less than 95% (P < 0.002), and only 40% had medium or dark staining (P < 0.001 compared to 90%, Fig. 3b).

As expected, Fluoro-Gold injections in the IC resulted in fluorescent motoneurons concentrated in the medial aspect of the DLN (Fig. 2a) whereas injections in the EUS resulted in fluorescent motoneurons concentrated in the lateral aspect of the DLN (Fig. 2b). However, the percentage of motoneurons with AR-immunopositive nuclei did not differ for these two pudendal subpopulations (main effect: P > 0.05). Although the percentage of EUS motoneurons with AR-positive nuclei tended to be lower than the percentage of AR-positive IC motoneurons (Fig. 3a), this difference was not significant, nor were any differences revealed between these two groups when estimates of the number of motoneurons with medium/dark AR-staining was compared (P > 0.05, Fig. 3b).

Because of the possibility that Fluoro-Gold spread between IC and EUS muscles could mask differences in AR expression between IC and EUS motoneurons, additional experiments were done that labeled IC and EUS motoneurons with different retrograde markers, Fluoro-Gold and Fluoro-Ruby, in the same animal. Such experiments verified the location of IC and EUS motoneurons in the DLN and their AR characteristics. As expected, IC and EUS motoneurons were located in predominantly medial and lateral parts of the DLN respectively (not shown). Moreover, quantitative analysis again revealed that about the same mean percentage of IC and EUS motoneurons have AR-positive nuclei [94 vs 91, respectively, based on n = 6 (half of the experiments were based on Fluoro-Gold-labeled IC motoneurons and Fluoro-Ruby-labeled EUS motoneurons and the other half were the reverse)].

**DISCUSSION**

To determine whether EAS and EUS motoneurons fail to enlarge in response to androgen treatment because they lack ARs, AR ICC was performed on spinal cord tissue in which specific pudendal motoneuronal subpopulations were retrogradely identified. As expected, virtually every motoneuron (96–97%) within the androgen-sensitive subpopulations (BC, LA, and IC) showed prominent AR immunostaining in their nu-

---

**FIG. 2.** Representative photomicrographs of AR-immunoreactive spinal cord sections showing Fluoro-Gold retrograde labeling of pudendal motoneurons in the DLN. Epifluorescent UV illumination at low magnification shows that IC motoneurons are concentrated in the medial aspect of the DLN (a) while EUS motoneurons are concentrated in the lateral aspect of the DLN (b). Bar, 200 μm (a–b). Higher power epifluorescent UV illumination reveals Fluoro-Gold labelling of individual ischiocavernosus (IC) (c) and external urethral sphincter (EUS) (d) motoneurons (arrows) while illumination with transmitted light reveals AR immunostaining in the nuclei of these same Fluoro-Gold-labeled IC (e) and EUS (f) motoneurons (arrows). Note the differences in AR staining density among the nuclei of IC and EUS motoneurons in (e). Bar, 100 μm (c–f).
This conclusion seems particularly appropriate for EUS motoneurons since the percentage of motoneurons with AR-positive nuclei in this group was no different than that for the IC motoneurons. Thus, while IC and EUS motoneurons differ in their androgen sensitivity (Collins et al., 1992), they present similar patterns of AR immunoreactivity. This fact is further underscored by two other observations. (a) When estimates are based on only those nuclei that show either medium or dark staining, eliminating the lightly stained nuclei, the average percentage dropped for both populations, but no difference was revealed. Since androgen-insensitive motoneurons belonging to the retrodorsolateral nucleus (RDLN) tend to show light AR staining in their nuclei (Freeman et al., 1995; Jordan et al., 1997), it was possible that differences in staining density would be observed that correlate with androgen sensitivity and that such differences might not be reflected in the total number of AR-positive motoneurons. (b) Moreover, when additional experiments were carried out that used Fluoro-Gold and Fluoro-Ruby to separately label EUS and IC motoneurons within the same animals, estimates of the number of EUS and IC motoneurons that had AR-immunopositive nuclei were virtually identical. Since motoneurons containing both Fluoro-Ruby and Fluoro-Gold were rare (only 1 double-labeled cell of 373 fluorescent cells), I conclude that Fluoro-Gold and Fluoro-Ruby successfully labeled specific subpopulations and that the results based exclusively on Fluoro-Gold are reliable.

While AR ICC failed to detect any difference between EUS and IC motoneurons, this technique did reveal a difference between EAS and BC/LA motoneurons. Significantly fewer EAS motoneurons were AR-positive and the staining was generally lighter. EAS motoneurons contained a disproportionate number of lightly stained (41% of AR-positive nuclei) nuclei compared to BC and LA motoneurons (9 and 4%, respectively). This result suggests that the apparent androgen insensitivity of this group might be accounted for by the fewer number of AR-positive cells and/or the larger proportion of AR-positive nuclei that were lightly stained. Hence, it is possible that some EAS motoneurons do indeed change in size in response to androgen, but that this response was not detected at the population level because it was masked by the large number of nonresponding (presumably AR-negative and possibly light, AR-positive) cells. Future experiments will address this possibility.
At first glance, the results based on AR ICC for EUS motoneurons seem to present a paradox, finding apparently equivalent AR expression in the face of reported differences in androgen sensitivity. One possible explanation is that the PG-21 antiserum failed to detect differences that might really exist in the AR complement of IC and EUS motoneurons. Another possible explanation for this paradox is that perhaps the androgen sensitivity of motoneurons is unrelated to whether or not the motoneurons have ARs. In other words, the effect of androgens on motoneuron size might be indirect, mediated through ARs somewhere else.

There is considerable precedence for the idea that the effect of androgens on SNB motoneurons is exerted indirectly via their target muscles (Araki, Harada, and Kuno, 1991; Fishman and Breedlove, 1992; Popper, Ulibarri, and Mycevych, 1992; Rand and Breedlove, 1995). For example, the length of SNB motoneuronal dendrites changes in response to adult androgens (Kurz et al., 1986), an effect that is mediated, at least in part, by androgen action on the target muscles (Rand and Breedlove, 1995). Thus, ARs within perineal target muscles could, in theory, account for why some pudendal motoneuronal subpopulations are insensitive to androgen. However, preliminary evidence based on AR ICC ($n = 3$) reveals that all five target muscles contain AR-immunopositive nuclei, including EAS and EUS muscles. Consequently, the androgen insensitivity of EAS and EUS motoneurons cannot be attributed to a lack of ARs in their target muscles. Although more detailed analysis may reveal differences in the AR complement among the various perineal targets, recent evidence suggests that ARs located within motoneurons, and not elsewhere, play a crucial role in the androgen-mediated change in motoneuronal cell body size.

While it has been unclear whether androgen acts directly or indirectly on SNB motoneurons to regulate their size (conflicting results from Rand and Breedlove, 1995 and Araki et al., 1991), recent results (Watson, Freeman, and Breedlove, 1996) provide compelling evidence which suggests that androgen probably acts directly on SNB motoneurons to control soma size. Watson and colleagues examined neonatally androgenized adult female rats which carried a defective copy of the AR on one X chromosome. Such animals are functionally mosaic for androgen sensitivity. Using such animals, these investigators find that only SNB motoneurons that express functional ARs, judged on the basis of AR immunostaining in their nuclei, change in cell body size in response to adult androgens. Within the same animal, SNB motoneurons that lacked nuclear ARs had smaller somata than the neighboring SNB motoneurons that possessed nuclear ARs, suggesting that the change in soma size of SNB motoneurons is a cell-autonomous response to androgen and that ARs within SNB motoneurons are crucial for this response. The present data extend this finding by indicating that while ARs may be necessary for this response, they are probably not sufficient.

Lubischer and Arnold (1995a,b) were the first to show a dissociation between AR expression and androgen sensitivity in SNB motoneurons. They found that SNB motoneurons axotomized 14 days after birth were insensitive to androgen in adulthood. That is, the size of previously axotomized SNB motoneurons was no longer influenced by adult androgens. Despite their lack of androgen sensitivity, such motoneurons express ARs (based on AR ICC) (Lubischer and Arnold, 1995b). Interestingly, it was not the axotomy, per se, that prevented later androgen sensitivity, since SNB motoneurons that were axotomized even a week later, on Day 21, retained their androgen sensitivity in adulthood (Lubischer and Arnold, 1995a). These results suggest that target muscles do indeed play an important role in conferring androgen sensitivity to SNB motoneurons and that there is a critical period for this effect.

The idea that some actions of steroid hormones require the presence of both their cognate receptors and accessory protein(s) is not new (Alberts, Bray, Lewis, Raff, Roberts, and Watson, 1989). Such factors are believed to facilitate the effects of other transcription factors, such as steroid receptors, but not to exert an effect on their own (Lindzey, Kumar, Grossman, Young, and Tindall, 1994). At least two different types of accessory proteins exist for steroid receptors, those that bind directly to DNA and those that bind to the hormone receptor itself (Lindzey et al., 1994). The role of accessory proteins as mediators of receptor activity comes primarily from studies based on the estrogen and glucocorticoid receptors. One example is heat-shock protein (hsp) 90, which binds to the estrogen receptor and is required for efficient receptor activation (Picard, Khursheed, Garabedian, Fortin, Lindquist, and Yamamoto, 1990; Landel, Kushner, and Greene, 1994). However, there is clear evidence that some androgen effects are mediated by interactions of its receptor and one or more accessory proteins. In the case of the androgen-regulated sex-limited protein gene, such an accessory protein(s) seems normally to reside in the nucleus and to enhance hormone function by binding directly to specific stretches of DNA that flank androgen response elements (Adler, Schellar, and Robins, 1993; Scarlett and Robins, 1995). Although some putative accessory proteins have been identified (reviewed by Horwitz, Jackson, Bain, Richer,
Takimoto, and Tung, 1996; e.g., Kupfer, Wilson, and French, 1994; Cavailles, Dauvois, L’Horset, Lopez, Hoare, Kushner, and Parker, 1993; Hong, Kohli, Trivedi, Johnson, and Stallcup, 1996; McNerney, Tsai, O’Malley, and Katzenellenbogen, 1996; Smith, Onate, Tsai, and O’Malley, 1996), there is considerable work to be done in determining the identity of such collaborators in a given hormone response in vivo and in understanding their role generally in steroid hormone action.

The percentage of AR-positive EAS motoneurons varied markedly from one animal to another (38–94%), raising the concern that the mean estimate for this subpopulation may be spuriously high due to the contaminating effects of nonspecific labeling of LA and BC motoneurons in some experiments. There are several reasons that make it unlikely that this variance represents nonspecific labeling of other pudendal groups. Animals that had a relatively high percentage of AR-positive EAS motoneurons did not also have an unusually high number of Fluoro-Gold-labeled cells in the SNB region, which might be expected if Fluoro-Gold had labeled some BC and/or LA motoneurons in addition to EAS motoneurons. Moreover, because of the location of the EAS muscle with respect to the other perineal muscles—the BC, LA, and IC are adjacent to one another and attach to the base of the phallus while the EAS is separated by a 2- to 4-mm space and rings the opening of the anus—it is unlikely that injections aimed at the EAS were picked up by LA and BC nerve terminals. Also, if the mean estimate for EAS motoneurons was spuriously high, then this should be reflected in a higher estimate for SNB motoneurons overall. However, the total percentage of AR-containing SNB motoneurons based on the present data (derived by collapsing the data across EAS, BC, and LA groups) matches an estimate (86%) previously reported for normal adult male rats without Fluoro-Gold (Jordan et al., 1997). While it is not entirely clear why AR expression is variable for EAS and not other pudendal subpopulations, the exact overlap of the present data for all SNB motoneurons with previously reported data suggests that the prevalence of AR expression in EAS motoneurons varies quite substantially from animal to animal and does not reflect spread of the retrograde marker.

In sum, the presence or absence of ARs in EAS and EUS motoneurons does not fully explain why these pudendal subpopulations fail to respond to androgens with a change in soma size. The lower proportion of AR-containing EAS motoneurons and the lighter density of immunoreactive staining may be factors that account for why EAS motoneurons, on average, are found to be insensitive to androgen (Collins et al., 1992). On the other hand, the high prevalence of AR-containing EUS motoneurons indicates that there are factors in addition to AR expression that determine whether androgen in adulthood can regulate the size of pudendal motoneurons.

ACKNOWLEDGMENTS

I thank Jane Lubischer for critical comments on earlier drafts and Gail Prins for the PG-21 antiserum. This work was supported by the National Science Foundation (IBN-9210229 and IBN-9309856).

REFERENCES


