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Steroid Modulation of Muscarinic Cholinergic and α_2-Adrenergic Receptor Density in the Nucleus Intercollicularis of the Japanese Quail

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Abstract

Androgen modulation of neurotransmitter receptor density was investigated in the nucleus intercollicularis (ICo) of male and female Japanese quail (*Coturnix coturnix japonica*). ICo appears to play an important role in the neural control of testosterone (T) dependent vocal behaviour. Two receptor types were investigated in this nucleus; muscarinic cholinergic receptors, labelled using [^{3}H]N-methyl scopolamine (NMS) as the ligand, and α_2-adrenergic receptors, labelled using [^{3}H]*p*-amino-clonidine (PAC) as the ligand. Changes in receptor density were assessed using *in vitro* quantitative autoradiography to ensure a high degree of anatomical specificity in the identification of any steroid effects. Gonadectomy was found to reduce the density of both [^{3}H]PAC bindings sites and [^{3}H]NMS in specific subregions of ICo. Gonadectomized animals treated with T had levels of receptor density similar to intact birds. However, the location within ICo of the effects of T was different for each ligand. [^{3}H]NMS binding was modulated only in a rostral subregion of the nucleus while changes in [^{3}H]PAC receptor density were found in the medial and lateral parts of ICo at a more caudal level. These changes in receptor density parallel changes in crowing frequency that are known to occur in males and females following castration and T treatment. The receptor systems may constitute a part of the neurochemical mechanism regulating vocal behaviour.

Introduction

The identification of discrete neural systems which mediate social behaviour has been made possible, at least in part, by the analysis of sexually dimorphic behaviours such as courtship, mating and singing. Sex steroids dramatically affect sex-typical behaviours and they are thought to exert their influence by activating sexually dimorphic neural networks which underlie this behaviour (Goy and McEwen, 1980). During the last two decades, the discovery of anatomical sex differences in the central nervous system of many species of birds and mammals, including man has provided important models to analyse how behaviour is organized at the neural level (DeVries *et al.*, 1983; Arnold and Gorski, 1984; Lakoski *et al.*, 1989; Balthazart, 1989). In particular, the discovery of the song control system by Nottebohm and his associates (Nottebohm *et al.*, 1976) has provided a very useful preparation in which the links between hormones, brain and behaviour can be directly established.

The mesencephalic nucleus intercollicularis (ICo) appears to be an important site mediating the activation of avian vocal behaviour. This is true in species exhibiting complex vocal behaviour, such as the 'songbirds' (i.e. members of the suborder Passeres) as well as in species with more simple vocal repertoires, such as species in the order Galliformes (e.g. chickens and quail) and the order Columbiformes (e.g. pigeons and doves) (Cohen, 1983; Seller, 1983, 1989). Electrical stimulation of this nucleus has been shown to elicit vocalizations in several avian species (Seller, 1983, 1989; Brown, 1971; Armitage and Seller, 1981) and bilateral lesions of the nucleus eliminate or reduce vocal activity (Brown, 1965; Cohen and Cheng, 1981). ICo also seems to be a critical neural site for the modulation of avian vocal behaviour by steroid hormones. For example, it has been demonstrated that ICo contains receptors for sex steroid hormones, such as testosterone and oestradiol, in every avian species in which receptors for these steroids have been studied (e.g. Martinez-Vargas *et al.*, 1976; Arnold *et al.*, 1976; Barfield *et al.*, 1978; Zigmond *et al.*, 1973; Lücke and Haase,

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1978; reviewed in Ball, 1990). In the Japanese quail (*Coturnix coturnix japonica*) receptors for testosterone, dihydrotestosterone, and 17β-oestradiol have all been demonstrated to occur in ICo (Watson and Adkins-Regan, 1989; Balthazart *et al.*, 1989). Functional studies have also implicated this nucleus as an important site for the action of sex steroids. For example, in the ring dove (*Streptopelia risoria*) steroid implants directed toward ICo in gonadectomized males and females were found to activate certain courtship vocalizations (Cohen and Cheng, 1981; Cohen, 1981) though a similar study failed to stimulate crowing in the domestic fowl (*Gallus domesticus*, Phillips and Barfield, 1977).

Androgens appear to be the primary class of steroid hormones activating certain vocalizations such as crowing in the Japanese quail (Adkins and Pneiwski, 1978; Schumacher and Balthazart, 1983; Alexandre and Balthazart, 1986; Wada, 1982). One of the many ways steroid hormones have been shown to modify neurotransmission and therefore possibly behaviour is through the modulation of neurotransmitter receptor density (McEwen *et al.*, 1986). In this communication we report on the modulation by testosterone (T) of muscarinic cholinergic receptors and α_2-adrenergic receptors in the quail ICo. In our previous studies of neurotransmitter receptors in this nucleus in the Japanese quail we have found that: (i) ICo contains a high density of α_2-adrenergic receptors as determined by quantitative autoradiography using [^{3}H]*p*-amino-clonidine (PAC) as the ligand (Ball *et al.*, 1989a); (ii) there is a sex difference in the density of these receptors in ICo which is more pronounced in the lateral portion of the nucleus in comparison to the medial portion (Ball *et al.*, 1989a); (iii) the α_2-receptors in ICo are primarily postsynaptic (Balthazart and Ball, 1989); (iv) ICo also contains muscarinic cholinergic receptors as labelled using the ligand [^{3}H]N-methyl scopolamine (NMS) and that this pattern of cholinergic receptor binding varies from the α_2-adrenergic receptor binding pattern as labelled with [^{3}H]PAC (Ball *et al.*, 1990); and (v) there is a dorsomedial subdivision in this nucleus identified by both α_2 receptor autoradiography and oestrogen receptor immunohistochemistry that resembles the dorsomedial part of the nucleus intercollicularis (DM) in the songbird ICo (Ball *et al.*, 1989b). This putative homology with the DM of songbirds is, however, based only on neurochemical criteria, and the connectivity of this subregion in ICo should be studied before definitive conclusions can be made. Given these facts concerning the regional heterogeneity of receptor density for both the muscarinic cholinergic system and the α_2-adrenergic system, we were especially interested in localizing in an anatomically precise way any effects of T on these receptor systems in this nucleus. We therefore employed the method of quantitative autoradiography for these investigations. These studies are a first step in elucidating how hormones modulate neurochemical functioning in this nucleus, future studies will concentrate on the possible relationship between these neurochemical changes and behavioural events.

Materials and methods

Experimental animals

Male and female quail (*Coturnix coturnix japonica*) were obtained from a local breeder in Belgium (F. Lefèvre, Boneffe). Birds were gonadectomized or sham-operated at the age of 4 weeks under metomidate (Hypnodil®) anaesthesia (15 mg/kg body weight, Janssen Pharmaceutica, Belgium) and then raised in groups without hormone treatment under a long photoperiod (16L:8D; light on a 0600 h) until the age of 5 weeks when intact birds normally reach sexual maturity (sexual maturity is observed between 30 and 40 days posthatch in male quail; Ottinger and Brinkley, 1979). A few days before the beginning of the hormone treatment (day one), they were isolated in individual cages under the same photoperiod. The mean body weight of the animals used in all experiments was approximately 200 g. Food (commercial flour for egg laying hens) and water were always available *ad lib.*

Experimental procedures

Birds were randomly assigned to experimental groups and 10 days after castration (day one of the experiment) were implanted with three silastic capsules (20 mm each; Dow Corning Silastic tubing number 602-252; 1.57 mm i.d., 2.41 mm o.d.) filled with T (Sigma T-1500) or left empty as control. Capsules were always preincubated overnight in saline solution to initiate steroid diffusion through the tube wall. They were placed subcutaneously in the neck region of the birds. The skin of the animals was first cleaned with ethanol 70°, a small incision (2−3 mm) was made in a region of the neck and the capsules were rapidly inserted under the skin with tweezers. We have shown previously that this treatment with T restores in castrates plasma levels of T which are similar to those found in sexually mature intact males (Balthazart *et al.*, 1987). This was confirmed here by the similar development of the cloacal gland in intact and in castrated T-treated males (see below). Six experimental groups were defined in this way: intact males (MI; $n = 6$), gonadectomized males (MGnx, $n = 5$), gonadectomized T-treated males (MGnx+T, $n = 5$), intact females (FI, $n = 8$) gonadectomized females (FGnx, $n = 7$) and gonadectomized T-treated females (FGnx+T, $n = 8$). Birds in both the intact and gonadectomized groups received empty implants. On day 25, the body weight of the birds was recorded and their cloacal gland area measured with a caliper (greatest length × greatest width). The animals were then killed by rapid decapitation. The brains were immediately dissected out of the skull and frozen on dry ice. On killing, completeness of castration and presence of the implants were checked. Data from birds with incomplete castration or in which implants could not be found were discarded before any analysis.

Quantitative autoradiography of α_2 adrenergic and muscarinic cholinergic receptors

Brains were mounted on specimen holders and sectioned (at −10 to −13°C) using a cryostat. Transverse sections (10 μm) were taken throughout the ICo of each bird. Alternate sections were labelled for α_2-adrenergic or muscarinic cholinergic receptors. The plane of sectioning was perpendicular to the ventral surface of the brain (the same plane as that used previously (Schumacher and Balthazart, 1987) and thus made an angle of about 10° with the vertical axis as defined in the stereotaxic atlas of the quail brain (Baylé *et al.*, 1974). Slices were thaw-mounted onto gelatin-coated microscope slides and stored for no more than 2 weeks at −30°C.

Muscarinic cholinergic receptors were labelled as described previously (Ball *et al.*, 1990; Ball *et al.*, 1989b). Slides were thawed and fan dried for 30 min. They were then placed in slide mailers containing the incubation medium. This medium consisted of 8 ml 50 mM potassium phosphate buffer (pH 7.4) containing [^{3}H]NMS 85 Ci/mmol, New England Nuclear, Boston, MA; incubation concentration: 1 nM, a potent muscarinic cholinergic antagonist. Nonspecific binding was estimated by incubating slides in a solution containing [^{3}H]NMS and the cold competitor, atropine, a muscarinic

antagonist (5 μM concentration). Sections were incubated for 1 h at room temperature. The incubation medium was then poured off and the sections washed in ice-cold buffer for 10 min.

α_2-Adrenergic receptors were labelled *in vitro* in a manner similar to that described for guinea-pig brain (Nock *et al.*, 1985) and which has been previously described and validated for a quail (Ball *et al.*, 1989a; Ball *et al.*, 1989b). Briefly, sections were thawed and dried with a fan at room temperature for 30 min. After a 30-min preincubation at room temperature in Tris−HCl buffer (50 mM Tris, pH 7.7), the slides were incubated for 60 min at room temperature with 2 nM [^{3}H] para-amino-clonidine, ([^{3}H]PAC, 45.6 Ci/mmol; New England Nuclear, Boston, MA). To define nonspecific binding, 10 μM phentolamine (phentolamine mesylate, obtained from Ciba-Geigy, Summit, NJ) was added to the incubation buffer. Following the incubation, sections were rinsed twice (5 min followed by 10 min) in ice-cold Tris-HCl buffer, dipped in ice cold distilled water and quickly dried with a fan. It has been shown that the binding observed in these conditions has a very high affinity in the nanomolar range, that it is saturable and exhibits a pharmacological specificity similar to that shown in mammalian forms (Ball *et al.*, 1989a).

Autoradiograms were produced by exposing Ultrofilm (LKB Inc., Gaithersburg, MD) to the labelled slices for 1 week ([^{3}H]NMS binding) or 6 weeks ([^{3}H]PAC binding). Film was developed for 5 min in Kodak D-19 developer, dipped in a stop bath (40 ml glacial acetic acid/l of distilled water) and fixed for 2 min in Kodak Rapid Fixer with hardener. All solutions were maintained at 20°C.

Images were analysed using the Drexel Unix-based Image Analysis System (DUMAS, Drexel University, Philadelphia, PA) that converted relative optical densities to moles bound per milligram of protein using a standard curve derived from coexposed tritium standards ([^{3}H] micro-scales, Amersham, Arlington Heights, IL). Wet weight was converted to protein using a standardized protein content of 10% as determined previously for the quail brain (Schumacher and Balthazart, 1987). These procedures have been validated in previous publications from our laboratories: the level of binding determined by scintillation counting of the bound radioactivity and by transformation of optical densities on autoradiograms is similar (Ball *et al.*, 1989a,b, 1990; Balthazart and Ball, 1989).

Statistics

In all cases the data were analysed using a two-way analysis of variance with sex of the bird as one factor and hormone condition (intact, gonadectomized; gonadectomized plus T) as the other factor. Differences were considered significant if the probability of attaining such a difference was less than or equal to 5%. In the text and in the figures all data are represented by their mean value and the associated standard deviation.

Results

Analysis of the measurements of the cloacal gland, a T sensitive structure (Sachs, 1967), demonstrated that the intact birds had reached sexual maturity by the time of killing (intact males: 320.8 ± 48.2 mm^2 females: 132.8 ± 21.8 mm^2 both of these are typical adult levels). These analyses also indicated that the gonadectomies were in all cases successful (MGnx: 34.9 ± 6.5 mm^2; FGnx: 34.9 ± 6.7 mm^2) and that T was successful in reinstating precastration cloacal sizes (MGnx+T: 309.9 ± 36.2 mm^2; FGnx+T: 222 ± 33.4 mm^2: $F_{2,33}$ for hormone treatment = 212.177 $p < 0.0001$). This induction by T also occurred in a sexually differentiated manner ($F_{2,33}$ for the interaction of sex and treatment = 32.417 $p < 0.001$; $F_{1,33}$ for sex difference = 91.772 $p < 0.001$).

The density of binding sites labelled by both [^{3}H]PAC and [^{3}H]NMS changed significantly as one progressed in the rostral-caudal plane. Both the qualitative and quantitative pattern of binding differed between the two ligands. Therefore it was necessary to assess steroid effects on receptor binding separately in different parts of the nucleus. In Figure 1 the qualitative pattern of receptor density for the two ligands is presented schematically in the transverse plane and the amount of binding detected at each level is presented in Figure 2. The values and the drawings are based on data collected from intact males, however, similar qualitative and quantitative patterns are apparent if all animals used in this study are combined. The highest density of cholinergic receptors was detected in the most rostral extent of ICo where the nucleus consists of a thick elongated band of cells whose long axis runs in the medial to lateral plane (referred to in the figures as the 'rostral band'). The nucleus mesencephalicus lateralis pars dorsalis (MLd) is not yet apparent at the most rostral extent of this band or it is only beginning to appear and is smaller than when sampled at a more caudal level as illustrated in Figure 1 panels A and B. As illustrated in Figure 1 panels A and B the dense band of NMS binding always appears ventral to MLd. As one samples caudally the dense band of binding disappears and ICo appears as two caps that cover both ends of the ovoid MLd. The NMS receptor density in both the lateral and the medial portions of the nucleus at this level is lower than that detected in the more anterior band of high binding, but it is higher than that measured in the adjacent MLd (Fig. 2). In the intact males, the density of NMS binding was significantly different in these four brain regions as determined by a one-way analysis of variance ($F_{3,20}$ = 151.093 $p < 0.001$).

The density of α_2-adrenergic receptors also changes as one samples throughout the rostral to caudal extent of the ICo. At the most rostral extent of the nucleus (Fig. 1 panel D) when it consists of an elongated band of cells without MLd being apparent or with MLd being at its most rostral extent and therefore very small, the density of [^{3}H]PAC binding is consistent throughout the nucleus in contrast to what was described above for [^{3}H]NMS binding which heavily labels an elongated ventral subregion of the nucleus. Caudal to this level when the nucleus surrounds the entire MLd the density of [^{3}H]PAC binding sites for both the medial and lateral portions of the nucleus is significantly higher than that observed at the more rostral level (see Fig. 1, panel E and Fig. 2). This density declines as one samples more caudally in both parts of the nucleus. The one exception to this decline in receptor density is the dorsomedial subregion of the nucleus that is apparent at this level (labelled 'DM' in Fig. 1, panel F). The binding in this small subregion of the nucleus equals that detected more rostrally (Fig. 2). The density of [^{3}H]PAC binding in MLd at both the rostral and caudal level of the nucleus illustrated in Figure 1 is low relative to the density of the surrounding ICo (Fig. 2). The density of binding sites in intact males is significantly different between these subregions of the ICO-MLd complex (one-way analysis of variance: $F_{7,39}$ = 29.148 $p < 0.001$). It should also be noted that, at the most caudal level illustrated in Figure 1, panels E and F, ICo as defined by [^{3}H]PAC binding differs from the pattern revealed using [^{3}H]NMS. The relatively low binding area in the centre of the ICo-MLd complex that generally corresponds to MLd is much larger at this level when one compares a [^{3}H]PAC autoradiogram with a [^{3}H]NMS autoradiogram. It thus appears that only the outer edges of ICo are being labelled by [^{3}H]PAC at this very caudal portion of the nucleus. Figure

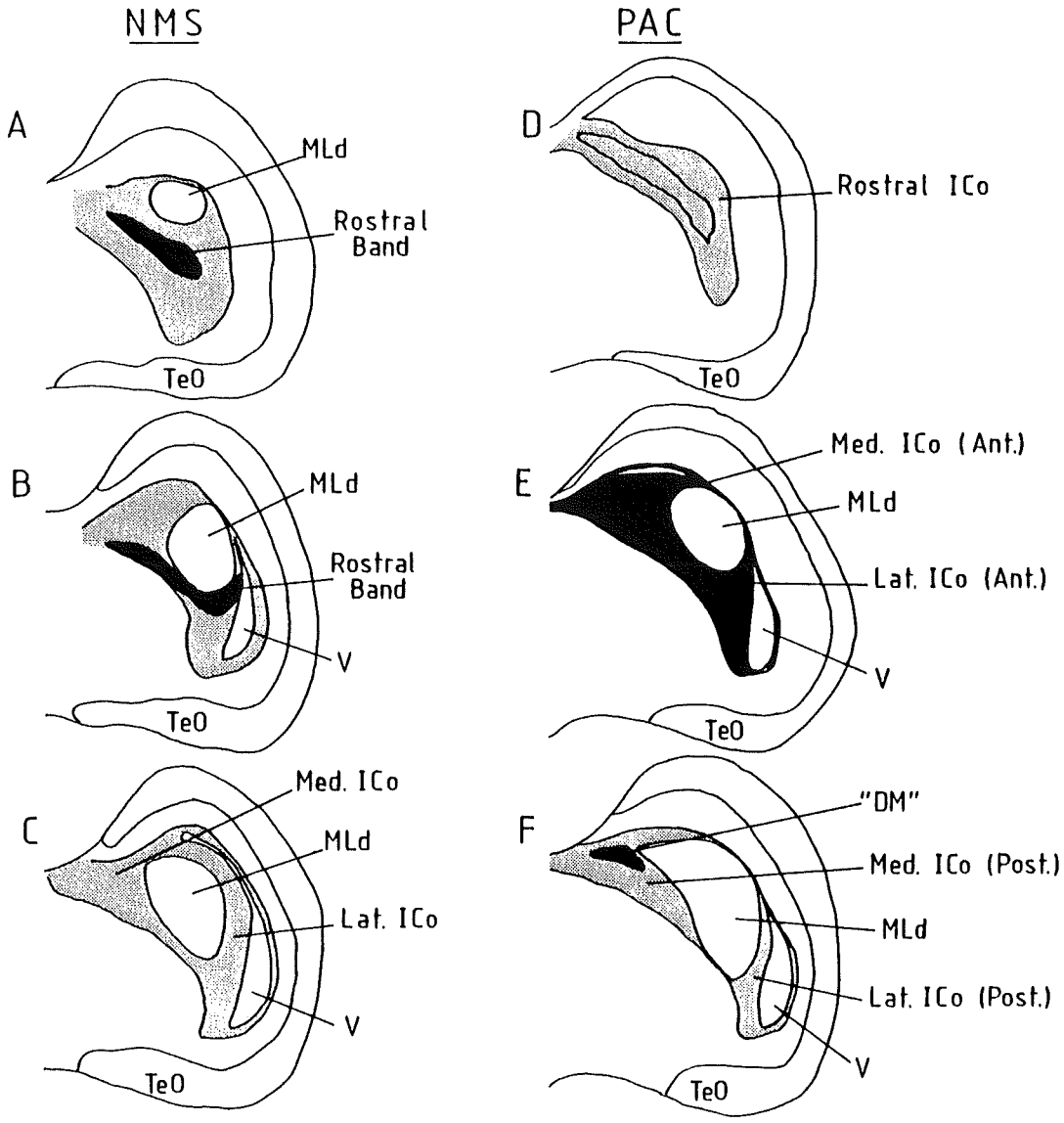

FIG. 1. Qualitative pattern of [^{3}H]NMS and [^{3}H]PAC binding in the nucleus intercollicularis (ICo) of the Japanese quail. Sections A–C and D–F are arranged in a rostral to caudal order. For a given ligand, the darkest regions correspond to the areas displaying heaviest binding. Ant: anterior, DM: dorsomedial part of the ICo, Lat: lateral, Med: medial, Mld: nucleus mesencephalicus lateralis pars dorsalis, Post: posterior, TeO: tectum opticum.

3 illustrates the density of [^{3}H]NMS binding in ICo in male and female quail that were intact, gonadectomized, or gonadectomized and treated with T. Figure 4 provides similar results for the sections labelled with [^{3}H]PAC. Table 1 contains a summary of the statistical analyses performed on the data derived from both the studies using [^{3}H]NMS and [^{3}H]PAC.

Figure 3 is divided into three panels that correspond to: (A) the heavy band of [^{3}H]NMS binding apparent in the most rostral extent of the ICo; (B) the medial portion of the nucleus sampled at a more caudal level identified as C in Figure 1; and (C) the lateral portion of the nucleus sampled at the same level. A significant effect of sex was not detected in any portion of the nucleus studied and a significant effect of treatment was only detected in the rostral band of heavy binding (Fig. 3 panel, A; Table 1). However, p values close to significance (i.e. $0.05 < p \leq 0.11$) were detected for treatment in the lateral ICo and for both treatment and sex in the medial portion of ICo (Fig. 3, panels B and C and Table 1).

Figure 4 is divided into 6 panels that represent the different subdivisions of ICo that were analysed for hormonal effects on [^{3}H]PAC binding. Panel A corresponds to the most anterior portion of the nucleus labelled D in Figure 1. Panels B and C correspond to the medial and lateral portions of ICo at the level of the nucleus labelled E in Figure 1. Panels E and F correspond to the binding detected in the lateral and medial portions of this nucleus at the most caudal level (labelled F in Fig. 1). Panel D refers to the 'DM' subregion of ICo illustrated in Figure 1 panel D. A significant effect of steroid treatment was observed in the medial portion of ICo at the level of ICo identified in Figure 1E (Fig. 4B, Table 1) and there was a significant effect of treatment and a significant interaction of treatment and sex in the lateral portion of ICo at the same level (Fig. 4C, Table 1). In the most anterior

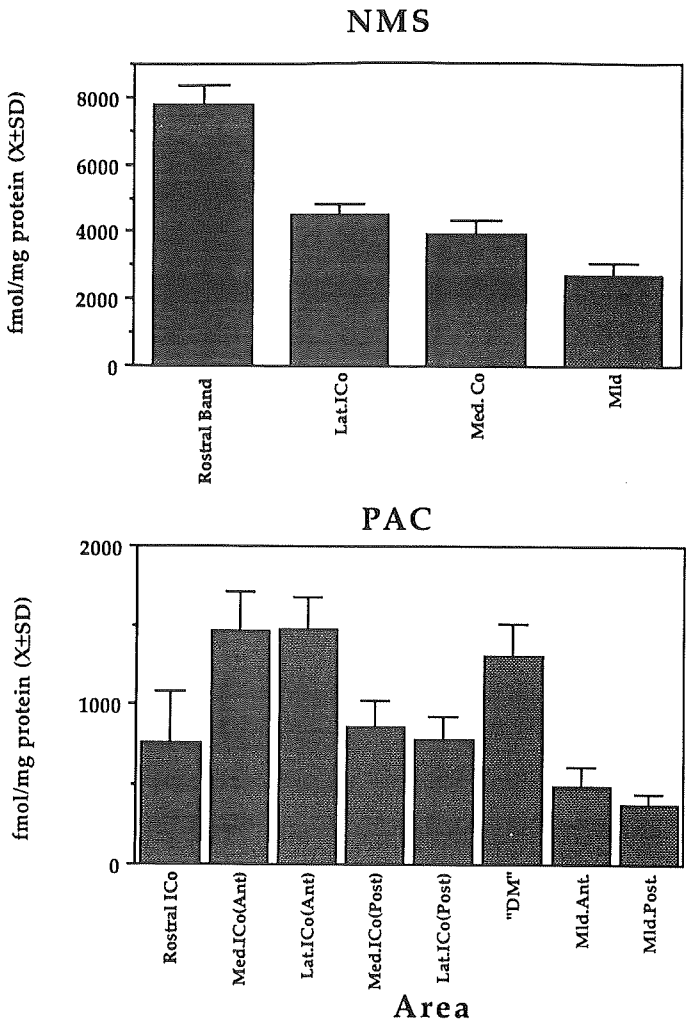

FIG. 2. Histogram showing the amount of [^{3}H]NMS and [^{3}H]PAC binding in different subregions of the nucleus intercollicularis (ICo) in male quail. Data are expressed as fmol/mg protein derived from coexposed standard curves. Ant: anterior, DM: dorsomedial part of the ICo, Lat: lateral, Med: medial, Mld: nucleus mesencephalicus lateralis pars dorsalis, Post: posterior, TeO: tectum opticum.

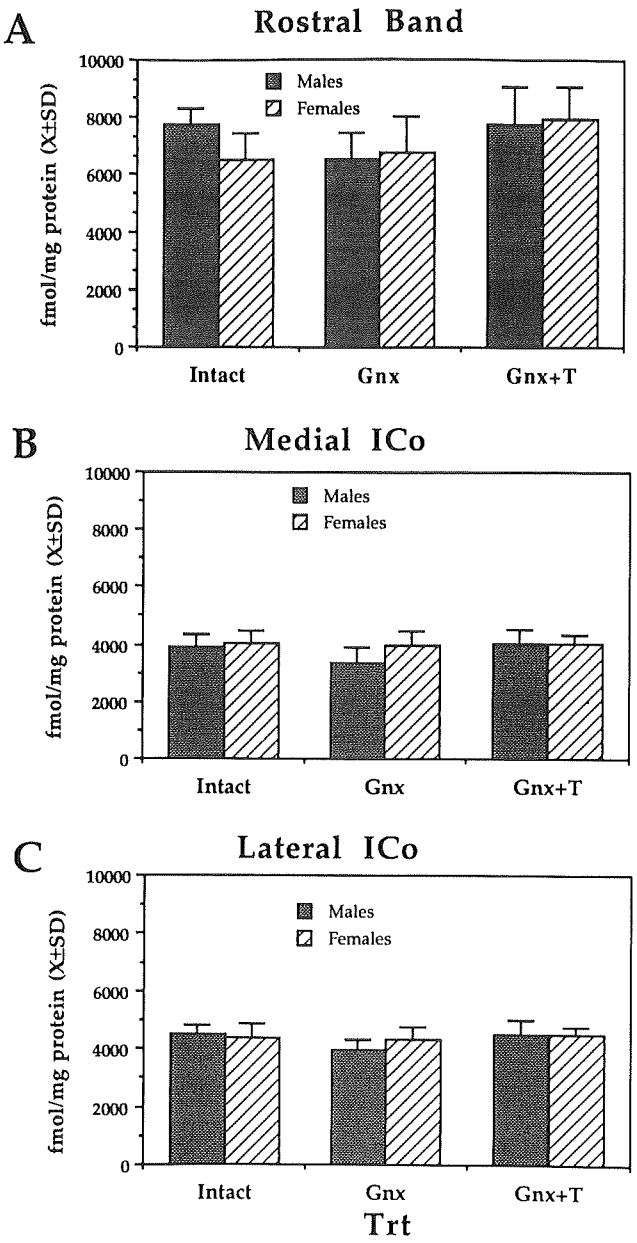

FIG. 3. Effect of the sex and hormonal treatment (Trt) on [^{3}H]NMS binding in subregions of the nucleus intercollicularis (ICo) in male and female quail. Birds of both sexes were studied as sexually mature animals (Intact) or after gonadectomy (Gnx) followed in some animals by a replacement therapy with testosterone (Gnx+T). Data were analysed by two-way analyses of variance (see Table 1).

portion of ICo there is a near significant effect (i.e. $0.05 < p \leq 0.10$) of hormonal treatment on [^{3}H]PAC binding.

Receptor density was quantified in MLd for both ligands on all autoradiograms where this nucleus appeared. In no case was any effect of gonadectomy or steroid treatment on the binding density of either [^{3}H]NMS or [^{3}H]PAC detected in this nucleus (Table 1).

Discussion

The α_2-adrenergic ligand, [^{3}H]PAC, and the muscarinic cholinergic ligand, [^{3}H]NMS, were each found to heterogeneously label in a unique way the mesencephalic ICo of the Japanese quail. The quantitative description of this heterogeneity confirms and extends the previous report on the binding pattern of these two ligands in this nucleus in the quail (Ball *et al.*, 1989b). In the present study it was also found that T modulated both [^{3}H]PAC and [^{3}H]NMS binding in ICo, but in each case the modulation was limited to discrete portions of ICo that were different for each ligand. In the case of [^{3}H]NMS, gonadectomy was found to decrease the receptor density and T treatment to reinstate the density exclusively in a subregion of the nucleus that corresponds to a rostral band of high muscarinic cholinergic receptor density (see Fig. 1A–B for the location of this band). In other more caudal parts of the nucleus (Fig. 1C) where the overall receptor density is lower, there was no apparent effect of gonadectomy or T treatment, though in both the medial and lateral portions of ICo in this more caudal region there was a nonsignificant trend for a similar decline in density after castration followed by a reinstatement with T treatment.

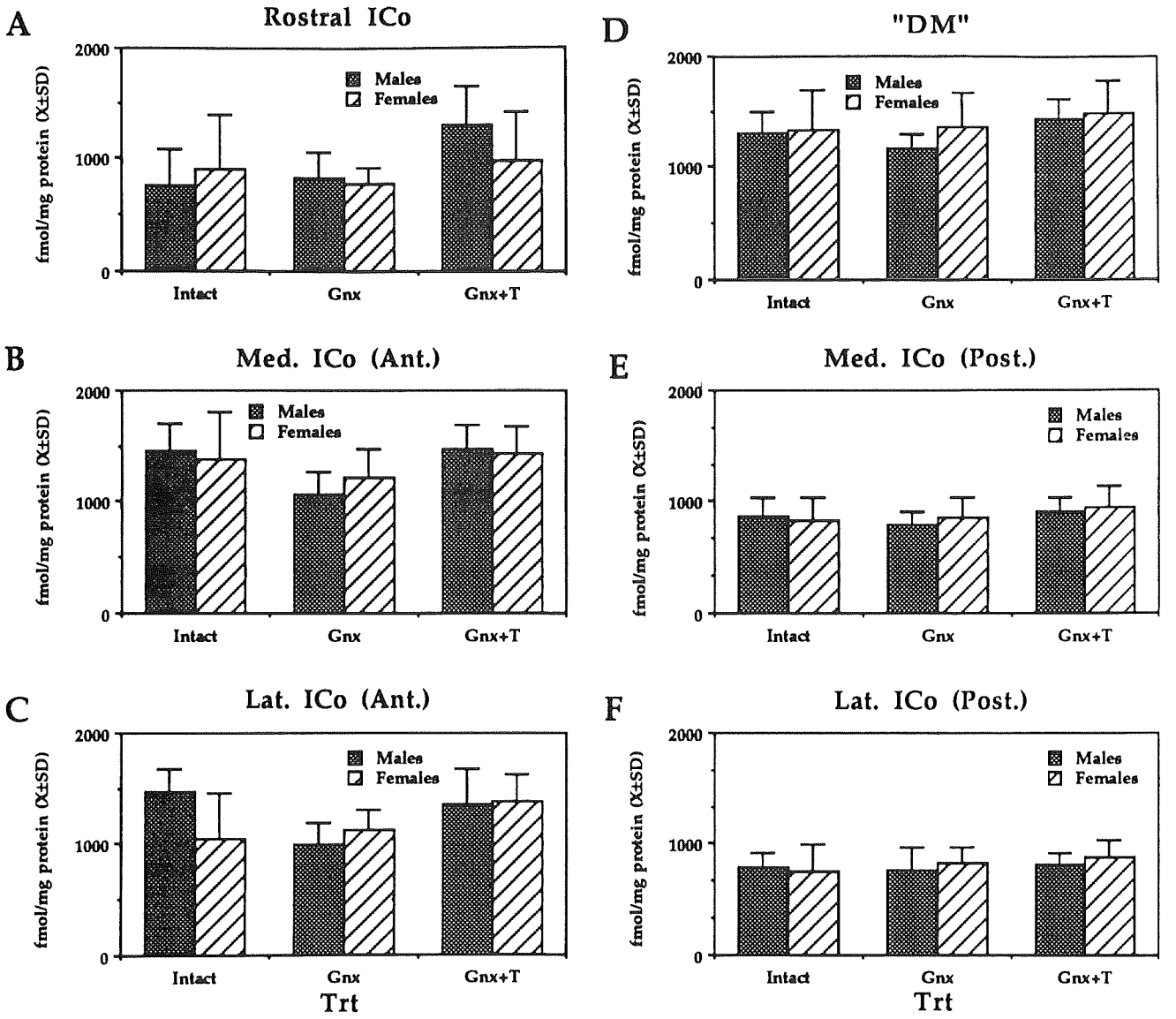

FIG. 4. Effect of the sex and hormonal treatment (Trt) on [^{3}H]PAC binding in subregions of the nucleus intercollicularis (ICo) in male and female quail. Birds of both sexes were studied as sexually mature animals (Intact) or after gonadectomy (Gnx) followed in some animals by a replacement therapy with testosterone (Gnx+T). Data were analysed by two-way analysis of variance (see Table 1). Ant: anterior, DM: dorsomedial part of the ICo, Lat: lateral, Med: medial, Mld: nucleus mesencephalicus lateralis pars dorsalis, Post: posterior.

[^{3}H]PAC binding was also found to exhibit a decline in density following gonadectomy. Binding levels were reinstated by T treatment. In this case the effects were limited to the medial and lateral portions of ICo at an intermediate level (see Fig. 1E). The level in the rostral-caudal plane where T is affecting [^{3}H]PAC overlaps to a certain extent, but in general it is more caudal than the area where similar effects of T were found on [^{3}H]NMS binding. More rostral and caudal portions of ICo where the overall density of α_2-adrenergic receptors is lower were not found to be effected by T treatment. The statistical analysis of the binding patterns in the lateral portion of ICo at this intermediate level also revealed a significant interaction of treatment with the sex of the subject. This significant interaction results from the fact that in the intact group male levels of α_2-adrenergic receptor density are substantially higher than the corresponding levels for the female (male intact: 1470 ± 197 fmol/mg protein; female intact: 1050 ± 407 fmol/mg protein). This confirms and localizes more precisely the previously reported sex difference in [^{3}H]PAC binding within the lateral part of the ICO (Ball *et al.*, 1989a). This sex difference appears to result from the different circulating levels of T in male and female quail in that castration decreases the level of binding in males to levels similar to those observed in females and T treatment of gonadectomized females increases the binding to levels characteristic of sexually mature males.

The efficacy of the gonadectomies and T-replacement was confirmed in all cases by the examination of the T-sensitive cloacal gland size. It has been shown in numerous studies that this gland declines after

TABLE 1. Statistical analysis of the effects of the sex of the birds and their hormonal condition (intact, gonadectomized, gonadectomized and T-treated) on the NMS and PAC binding in subregions of the nucleus Intercollicularis

Brain Area	Sex effect		TRT effect		interaction	
	F	p	F	p	F	p
NMS						
Rostral band	0.570	ns	4.005	0.028	1.901	ns
Med. ICo	2.715	0.109	2.393	0.108	1.719	ns
Lat. ICo	0.235	ns	2.727	0.081	1.269	ns
MLd	0.490	ns	0.664	ns	0.933	ns
PAC						
Rostral ICo	0.386	ns	3.108	0.061	1.109	ns
Med. ICo (Ant.)	0.019	ns	4.468	0.020	0.512	ns
Lat. ICo (Ant.)	0.889	ns	3.555	0.041	3.453	0.045
MLd (Ant.)	0.621	ns	1.628	ns	1.062	ns
Med. ICo (Post.)	0.186	ns	1.188	ns	0.296	ns
Lat. ICo (Post.)	0.208	ns	0.618	ns	0.285	ns
MLd (Post.)	0.151	ns	1.494	ns	1.418	ns
DM	1.154	ns	1.851	ns	0.375	ns

Data were analysed by two-way analyses of variance with the sex of the birds and their treatment (TRT) as factors. The table reports the F values for the effects of each factor and of their interaction and the associated probability values (p). ICo: nucleus intercollicularis; Med: medial; Lat: lateral; Ant: anterior; Post: posterior; MLd: nucleus mesencephalicus lateralis pars dorsalis; DM: dorso-medial part of the nucleus intercollicularis.

gonadectomy to sizes in the range of those reported in the present study (e.g. Balthazart *et al.*, 1983; Balthazart *et al.*, 1987). Physiological levels of T (i.e. levels typical of sexually mature and reproductively active males) are required to induce gland sizes similar to those reported in the T treated groups. It has also been established that physiological levels typical of reproductively active males are observed following the implantation of 40 or 60 mm silastic capsules filled with T similar to those used here (Balthazart *et al.*, 1983, 1987; Schumacher and Balthazart, 1986). Plasma levels observed following the administration of implants of this size are similar in males and females (Balthazart *et al.*, 1987; Schumacher and Balthazart, 1986), but the cloacal gland is still different between the sexes because of differential sensitivity to steroid action that results from early organizational effects of steroids. Thus all effects of T on receptor binding that were detected in subregions of ICo closely parallel the changes in peripheral androgen levels.

It also appears that males and females are equally able to respond to steroid treatment and that there is no sex difference in the central sensitivity to steroid action in ICo in contrast to the case for the peripheral T-sensitive structure, the cloacal gland. These T effects on receptor binding mirror the effects of T on crowing in that females do not normally crow but can be induced to crow if they are treated with supraphysiological doses of T (Adkins and Pniewski, 1978; Schumacher and Balthazart, 1983; Balthazart *et al.*, 1983; Adkins and Adler, 1972).

Steroid hormones have been shown to modulate both cholinergic and α_2-adrenergic neurotransmitter receptor density in a variety of hypothalamic nuclei of rats and guinea-pigs. In these studies, it was also found that the steroid effects were limited to rather small subdivisions of the nucleus. These studies, and the present one, emphasize the importance of maximizing anatomical resolution when studying hormone effects on brain neurochemical functioning. The changes in neurotransmitter receptor binding induced by steroids in the mammalian brain have been best characterized for the action of 17β-oestradiol in the ventromedial hypothalamic nucleus of the rat where the modulation appears to be related to the induction of female mating behaviour. The changes in neurotransmitter binding presumably reflect changes in the actual concentration of the receptors. They might, however, also have been brought about by steroid-induced changes in neuronal growth (DeVoogd and Nottebohm, 1981). Changes in tissue density affect autoradiographic efficiency and therefore differences in optical density might then be due to the effects of T on tissue density rather than to true differences in neurotransmitter binding. This possibility cannot be totally excluded, but it seems quite unlikely considering that we have never detected any effects of treatments on the binding of both ligands (NMS and PAC) in a given area. For the same reason, it seems unlikely that the receptor density changes might be due to changes in the total size of brain regions resulting in a variation of density of a constant amount of receptor. It seems that the steroid modulation of neurotransmitter receptor density represents one general way in which steroids modulate neurotransmission to produce behavioural effects (McEwen *et al.*, 1986; Dohanich *et al.*, 1985). The role of the muscarinic cholinergic and the α_2-adrenergic receptor systems in the control of vocal behaviour in quail is still poorly understood. However, both of the receptor systems have been implicated in the control of vocalizations in other avian species and in mammals (Rossi *et al.*, 1983; Panksepp, 1986; Newman, 1988). Further studies are now needed to elucidate the functional significance of this anatomically circumscribed effect of steroids on neurotransmitter receptor binding in the quail ICo.

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Abbreviations

DM	dorsomedial part of the nucleus intercollicularis
ICo	nucleus intercollicularis
FGnx	gonadectomized female
FGnx+T	gonadectomized female treated with testosterone
FI	intact female
MGnx	gonadectomized male
MGnx+T	gonadectomized male treated with testosterone
MI	intact male
MLd	nucleus mesencephalicus lateralis pars dorsalis
NMS	N-methyl scopolamine
PAC	para-amino-clonidine
T	testosterone

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