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Distribution of α_2 -adrenergic receptors in the brain of the Japanese quail as determined by quantitative autoradiography: implications for the control of sexually dimorphic reproductive processes

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With the use of [³H]*p*-aminoclonidine (PAC), α_2 -adrenergic binding sites were mapped in the brain of the Japanese quail (*Coturnix coturnix japonica*). The sites were labeled with the use of in vitro quantitative autoradiography. Special attention was given to areas implicated in the control of sexually dimorphic reproductive processes including sexual behavior. Preliminary competition experiments found that [³H]PAC binding on tissue sections exhibited a pharmacology appropriate to the α_2 receptor. Binding sites were found to be heterogeneously distributed throughout the brain. Some of the highest levels of specific binding were found in several areas regulating reproductive function such as the preoptic area, the supraoptic nucleus, the infundibulum, and the medial mammillary nucleus of the infundibulum. [³H]PAC labeled precisely the morphologically dimorphic preoptic medial nucleus but no sexual dimorphism in density of receptor binding was identified. However, dimorphism in density of receptor binding was identified in two areas: the medial mammillary nucleus and the mesencephalic intercollicular nucleus. The former area appears to be involved in the regulation of gonadotrophin secretion and the latter area has been implicated in the control of vocal behavior. These neurochemical dimorphisms may contribute to the regulation of two sexually dimorphic reproductive processes, gonadotropin secretion and courtship vocalizations.

INTRODUCTION

In the Japanese quail (*Coturnix coturnix japonica*), as in many other avian species and in mammals, a number of reproductive processes are sexually differentiated²¹. For example, male-type copulatory behavior in quail is readily elicited in castrated males by testosterone (T) treatment but cannot be activated in females even after the administration of pharmacological doses of the hormone^{2,4,5}. Other reproductive processes show quantitative differences between the sexes. For example, crowing is more frequent in males than in females even after treatment with the same dose of T⁵, and plasma levels of

the two gonadotropin hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are higher in males than in females irrespective of their gonadal condition (i.e. intact, gonadectomized, or gonadectomized with steroid replacement^{5,48,57}).

These reproductive events are controlled by the brain and in a number of cases discrete nuclei have been identified that play a critical role in mediating these responses. One way in which steroids modify behavior is through changes in neurochemical activity. The α -adrenergic system has been implicated in the control of many reproductive processes in mammals and birds. Evidence accumulated over the past 10 years, suggests that norepinephrine (NE) acting

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through α_1 - and α_2 -receptors, stimulates LH secretion in a variety of mammals and avian forms^{12,43,46}. Several lines of neurochemical evidence derived primarily from studies of laboratory mammals and birds suggest a close relationship between gonadotropin-releasing hormone (GnRH)-containing neurons and adrenergic terminals in the preoptic area of which provides anatomical evidence for the control of gonadotropin secretion by the adrenergic system^{29,46}. The role of NE in the control of sexual behavior is more controversial. A number of pharmacological or lesion studies conducted primarily with rats or guinea pigs suggest that NE plays a stimulatory role in the control of behavior but the magnitude of these effects is usually small, their generalization has been questioned, and a number of inhibitory effects have also been reported^{17,33}. In birds the effects of NE on male reproductive behavior have been investigated in only two species: the ring dove (*Streptopelia risoria*) and the Japanese quail. In both cases, the pharmacological treatments point to an inhibitory action of noradrenergic transmission on behavior^{7,10,11}. In the ring dove at least, the effect seems to be mediated by both the α_1 - and the α_2 -adrenergic system¹⁰. For example, the 'bow-coo' a male courtship call can be inhibited by central administration of both the α_2 agonist clonidine and the α_1 agonists methoxamine and phenylephrine¹⁰.

The neurochemistry of the steroid sensitive nuclei involved in the control of reproductive processes is poorly understood. The neuroanatomic characterization of the various neurochemical substances acting in these regions is an essential prelude for functional investigation of the neurochemical processes underlying reproduction. Neurotransmitter functioning involves a signal (transmitter release) and a receiver (receptor). In the quail, studies have been initiated and are continuing on transmitter synthesis and release^{38,39}. Neurotransmitter receptors are known to be affected by steroids. For example, in guinea pigs, α -adrenergic receptors are modulated by 17 β -estradiol in brain areas that mediate the effects of the steroid on lordosis behavior²⁵. Quantitative autoradiography is a useful technique to study these interactions because it simultaneously provides information on the specific anatomic localization of receptor subtypes and gives insight into quantitative

changes. In this communication, we describe the use of quantitative autoradiography to map and characterize the binding sites of an α_2 -adrenergic ligand, *p*-aminoclonidine in the quail brain. Special emphasis is placed on the identification of sexual dimorphisms in binding and on the distribution of receptors in areas involved in reproduction in order to better understand the neurochemical substrate mediating sexually dimorphic behaviors.

MATERIALS AND METHODS

Birds

Sexually mature male and female Japanese quail were used for these studies. The birds were purchased at the age of 4–5 weeks from a local breeder in Belgium (F. Lefevre, Boneffe-Eghezee). At the age of 7–8 weeks males were tested twice for the presence of sexual behavior during a standard 5 min presentation with a receptive female. All males which failed to show cloacal contact movements (copulation) during these tests were excluded from the experiment. At that time most females had started to lay eggs regularly and females failing to do so were also excluded. Throughout their life at the breeding colony and in the laboratory, birds were exposed to a photoperiod simulating long days (16L:8D). Food and water were always available ad libitum.

At 8 weeks of age birds were killed by decapitation and their brains were rapidly dissected out of the skull and frozen on powdered dry ice. Brains were stored at -70°C until they were processed.

Drugs

[³H]*p*-amino-clonidine ([³H]PAC; 45.6 ci/mmol; New England Nuclear, Boston, MA) was the ligand selected to label α_2 -adrenergic receptors. Phentolamine HCl or mesylate (obtained from Ciba-Geigy, Summit, NJ) was used to define non-specific binding. For the specificity experiments, (–)-norepinephrine (–NE, arterenol bitartrate, Sigma, St. Louis, Mo.), (±) norepinephrine (±NE, arterenol HCl, Sigma), (–)-epinephrine (–E, Sigma), clonidine (CLO, clonidine HCl, Sigma), yohimbine (YOH, yohimbine HCl, Sigma), phenylephrine (PHE, phenylephrine HCl, Sigma), prazosin (PRA, prazosin HCl obtained from Pfizer Laboratories, New

York), and isoproterenol (ISO, isoproterenol bitartrate salt, Sigma) were utilized.

Quantitative receptor autoradiography

Brains were mounted on specimen holders and sectioned (at -10 to -13 °C) using a cryostat. Coronal sections ($10\text{ }\mu\text{m}$) were taken every $100\text{ }\mu\text{m}$ through the preoptic area (POA) and every $300\text{ }\mu\text{m}$ through the rest of the brain. The sampled area extended throughout the hypothalamus ranging from a point 2 mm rostral to the POA to the level of the oculomotor nerves. The plane of sectioning was perpendicular to the ventral surface of the brain (the same plane as that used previously⁵⁰) and thus made an angle of about 10° with the vertical axis of the stereotaxic atlas of the quail brain¹³. Slices were thaw-mounted onto gelatin-coated microscope slides and were stored for no more than two weeks at -30 °C.

α_2 -Adrenergic receptors were labeled in vitro in a manner similar to that described for guinea pig brain³⁴. 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 slices were incubated for 60 min at room temperature with 2 nM [^3H]PAC. To define non-specific binding, $10\text{ }\mu\text{M}$ phentolamine was added to the incubation buffer. Following the incubation, sections were rinsed twice (5 followed by 10 min) in ice-cold Tris-HCl buffer, dipped in ice-cold distilled water and quickly dried with a fan.

Autoradiograms were produced by exposing Ultrafilm (LKB Inc., Gaithersburg, MD) to the labeled slices for 6 weeks. Film was developed for 5 min in Kodak D-19 developer, dipped in a stop bath (40 ml glacial acetic acid/liter of distilled water) and fixed for 2 min in Kodak Rapid Fixer with Hardener. All solutions were maintained at 20 °C.

Autoradiograms were analyzed using the Drexel Unix-based iMage Analysis System (DUMAS) that converts optical densities to mol bound/mg protein using a standard curve derived from co-exposed tritium standards (^3H microscaler, Amersham). Wet weight was converted to protein using a standardized protein content of 10% as determined previously for the quail brain⁵⁰. The brains of 8 males and 8 females were analyzed. For each bird, 2–15 readings

were taken in each region. Brain areas were identified by reference to the labeled sections which were stained with thionin or Cresyl violet after the autoradiographic procedure was completed. Based on previous studies in mammals²⁷, it was expected that sex differences, if they occur at all, would be limited to steroid-sensitive areas. Following the procedures of other authors^{25,27}, the density of binding in the two sexes was compared for each brain area by a two-tailed Student *t*-test. Although this procedure increases the chances of a type I error, from the perspective of the biology of the system, this is a defensible method because it allows for the detection of neuroanatomically discrete sex differences in receptor binding. Using a global analysis of variance is technically preferable from a statistical point of view, but this would increase the risk of a type II error to such a degree that it would be biologically inappropriate⁵⁵.

Binding characteristics

Because [^3H]PAC binding has been studied primarily in mammals, a number of preliminary experiments were carried out to assure that this ligand binds with similar characteristics in birds. Assays for those experiments were conducted as described for autoradiography except that at the end of the assay the amount of radioactivity bound per slice was determined by scintillation counting. Brain slices were sectioned from minced and molded tissue prepared from approximately 75 hypothalami — a brain area that contains high levels of [^3H]PAC binding sites in birds. These assay modifications are standard for preliminary autoradiographic studies and serve to circumvent the time delay associated with the generation of autoradiograms and to minimize variance in receptor density between slices.

To determine the affinity (apparent dissociation constant, k_d) and saturability (maximum number of binding sites, B_{max}) of [^3H]PAC binding, brain-mash sections were incubated with a range (0.1–8.0 nM) of [^3H]PAC concentrations. Total and non-specific binding were determined by scintillation counting. In addition, to test whether there are major differences in binding between the brain mash preparation and the brain slices, we also determined by autoradiography the K_d and B_{max} in several selected brain nuclei of one male.

To evaluate the specificity of [3 H]PAC for α_2 -receptors, sections of brain mash were incubated with 4 nM [3 H]PAC in the presence of two different concentrations (10^{-5} and 10^{-7} M) of several unlabeled compounds. A more detailed analysis of the effects of norepinephrine and epinephrine (10^{-9} to 10^{-5} M) on [3 H]PAC binding was also carried out. In order to protect the competitors from degradation, incubations were carried out in buffer containing 0.01% ascorbic acid, 10 mM MgCl_2 and 100 μM pargyline.

RESULTS

Binding characteristics

The results of preliminary studies indicated that [3 H]PAC binds to sites in the avian brain with characteristics similar to those previously described for mammals. In competition experiments with sections prepared from minced and molded hypothalami (brain mash), agonists competed for [3 H]PAC binding sites with an order of potency characteristic of α receptors; i.e. clonidine > (-)-epinephrine > (-)-norepinephrine > ($\pm$)-norepinephrine $\geq$ phenylephrine $\geq$ isoproterenol. In addition, the α_2 -antagonist yohimbine was more potent than the α_1 -antagonist prazosin (Fig. 1). Thus, [3 H]PAC appears to bind selectively to α_2

receptors in the avian brain as it does in the mammalian. In addition, the K_d and B_{max} for the dorsomedial nucleus of the anterior thalamus, the infundibulum, and the ventral archistriatum were similar to those derived from experiments with brain mash (Fig. 2) and those previously reported for mammals^{34,56}. The apparent affinity in the ventromedial nucleus (VMN) was about 3 times lower, however, this nucleus only appeared in a limited number of sections thereby possibly preventing an accurate determination of the binding characteristics. Slices of brain mash were 15 mm in diameter and 10 μm thick which corresponds to approximately 0.18 mg protein (based on a 10% protein content). The B_{max} as determined by β -counting of the brain 'mash' sections is thus equal to 149 fmol/mg protein. This value is in reasonable agreement with results obtained by autoradiography in that the 'mash' includes areas with high and low amounts of binding.

Distribution of α_2 receptors in the brain of male and female quail

Representative autoradiograms of α_2 -receptor binding are shown in Fig. 3 and the density of α_2 -receptors in selected brain areas are given in Fig. 4. α_2 -Receptors are distributed heterogeneously in the quail brain with nearly a 10-fold difference between the density of α_2 -receptors in areas with low con-

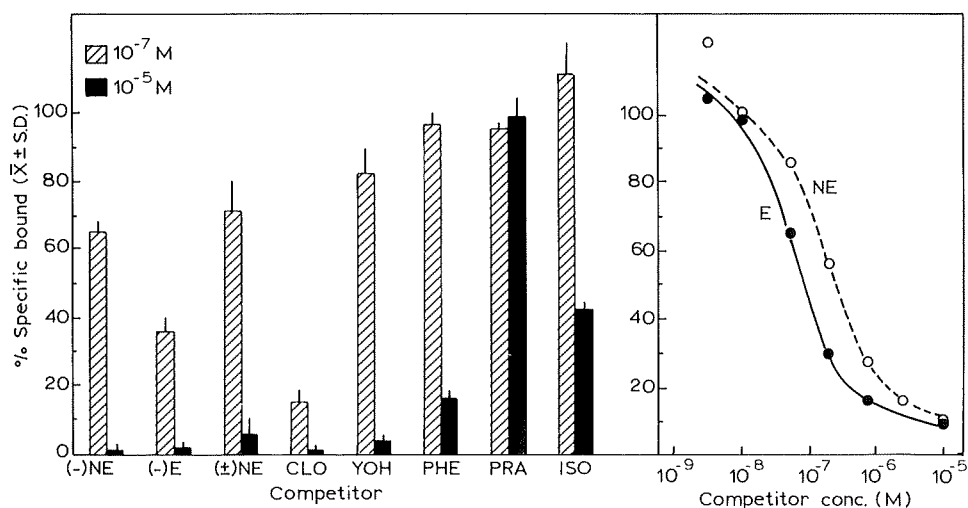


Fig. 1. Characterization of α_2 specificity of [3 H]PAC binding in quail brain tissue sections. Left panel presents histograms for a variety of drugs at two concentrations. Drugs are arranged from the left to the right according to their known affinity for the different adrenergic receptor subtypes. The right panel presents a competition curve for [3 H]PAC with a range of concentrations of epinephrine and norepinephrine.

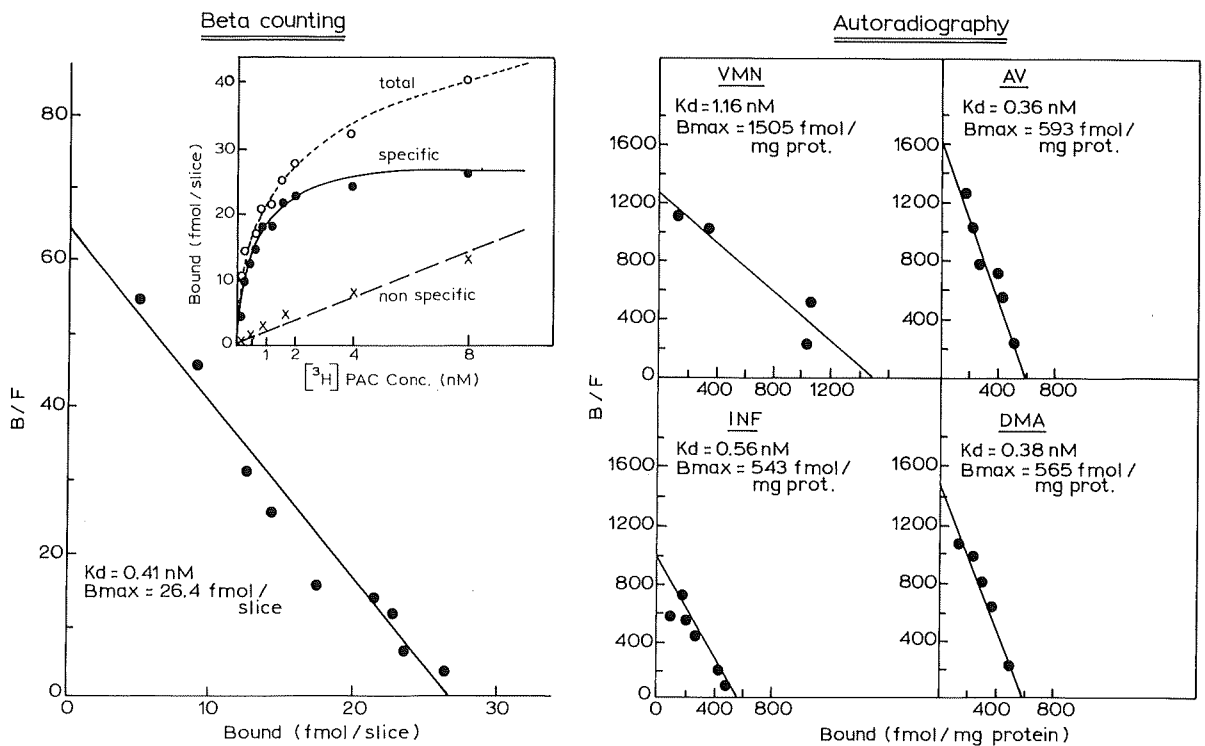


Fig. 2. Saturation analysis of $[^3\text{H}]$ PAC binding as determined by β -counting (left panel) or on autoradiograms (right panel) using procedures described in the text. In the left panel there is a Scatchard plot of the data presented in the boxed insert. In the right panel are individual Scatchard plots of characterizing the binding in 4 individual nuclei.

centrations such as cerebellum and those with high concentrations (Fig. 4). Dense binding was consistently observed in several hypothalamic and limbic structures including the medial preoptic nucleus (POM), ventromedial nucleus of the hypothalamus (VMN), and medial mammillary nucleus (MM). In the area of the supraoptic nucleus (SON), a dense band was observed which includes medial and lateral portions of the supraoptic region, the medial supra-chiasmatic nucleus and a ventro-lateral portion of the preoptic region. This entire band is labeled SON in both the plates and the figures showing relative binding density. The pattern of the density of α_2 -receptor binding outlined, in a manner identical to that seen in Nissl-stained sections, other brain areas such as the dorsomedial nucleus of the anterior hypothalamus (DMA) and nucleus intercollicularis (ICo) (see Figs. 4 and 6).

Sex differences in $[^3\text{H}]$ PAC binding

The pattern of $[^3\text{H}]$ PAC binding corresponds exactly with the sexually dimorphic POM indepen-

dently defined by stains for Nissl substance (i.e. thionin or Cresyl violet). This is illustrated in Fig. 5 which shows two paired autoradiograms and thionin stained-adjacent sections, one from the rostral portion of the nucleus and one from the caudal. The overlap between $[^3\text{H}]$ PAC binding and the Nissl stain is consistent along the entire anterior to posterior extent of the nucleus. In cases where an asymmetry between the left and right sides in the Nissl stains was observed, this was reflected in the adjacent autoradiograms. The density of α_2 -receptors in POM was not significantly different between females and males (Fig. 4).

Sex differences in the density of α_2 -receptors were identified in two brain areas, the medial mammillary nuclei of the tuberal region (males = 1790 ± 340 ; females = $1440 \pm 150 \text{ fmol/mg protein}$, mean $\pm$ S.D.; $t = 2.45$, $2P < 0.05$; see Fig. 6,7) and the intercollicular nucleus where the density of receptor binding was higher in males (830 ± 170) than females (620 ± 120 ; fmol/mg protein mean $\pm$ S.D.; $t = 2.39$, $2P < 0.05$). In a preliminary analysis of

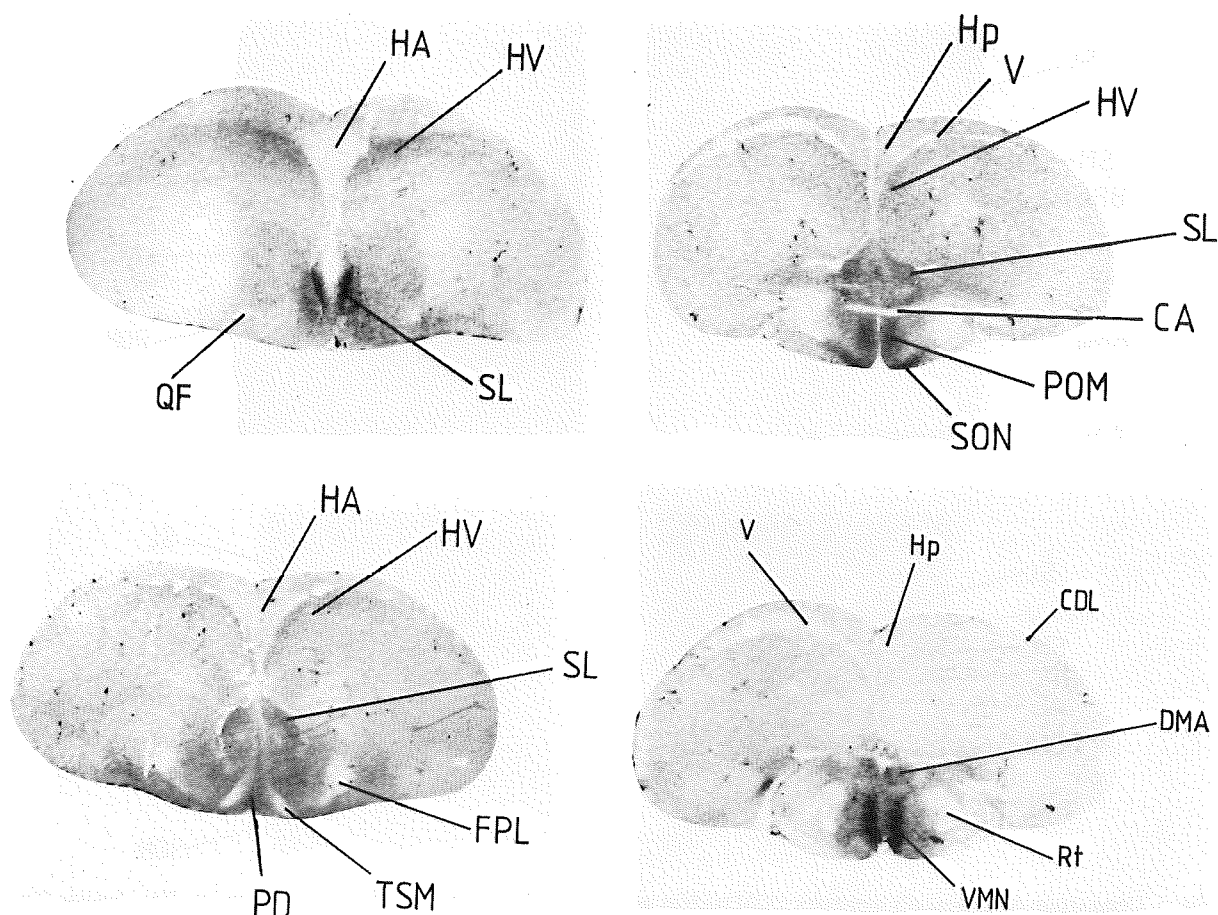


Fig. 3. Representative autoradiograms of [^3H]PAC binding in the quail brain. Examples selected are from females. The two pictures on the left are rostral to the two pictures on the right.

binding, we treated the ICo as a homogeneous structure. However, more detailed examination revealed that [^3H]PAC binding in ICo was not homogeneous; it tended to decrease from its medial to lateral extent especially in females and from the rostral to caudal extent in both sexes. Values for medial and lateral ICo were thus re-examined separately. This showed that the concentration of α_2 -receptors was higher in males than in females both in the medial and lateral part but the difference reached significance only in the lateral part (see Fig. 4). In order to insure that the sex difference identified in [^3H]PAC binding was not a result of males and females being sampled at different levels, we ran a two-way analysis of variance (ANOVA) that simultaneously tested binding differences by sex and by position in the rostrocaudal plane. ICo together with the mesencephalicus lateralis pars

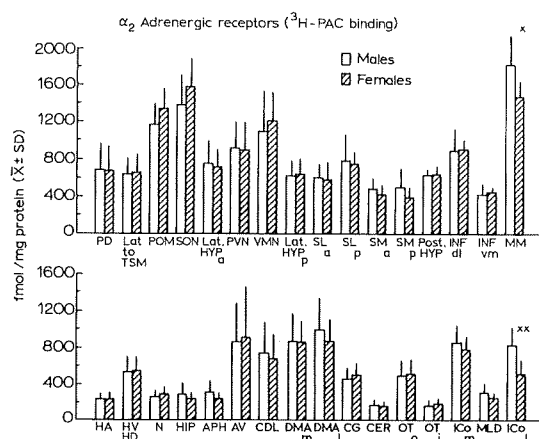


Fig. 4. Histograms showing the amount of [^3H]PAC binding in autoradiograms of 32 brain areas in male ($n = 8$) and female ($n = 8$) Japanese quail. Data are expressed as fmol/mg protein derived from coexposed standard curves (see Materials and Methods for details).

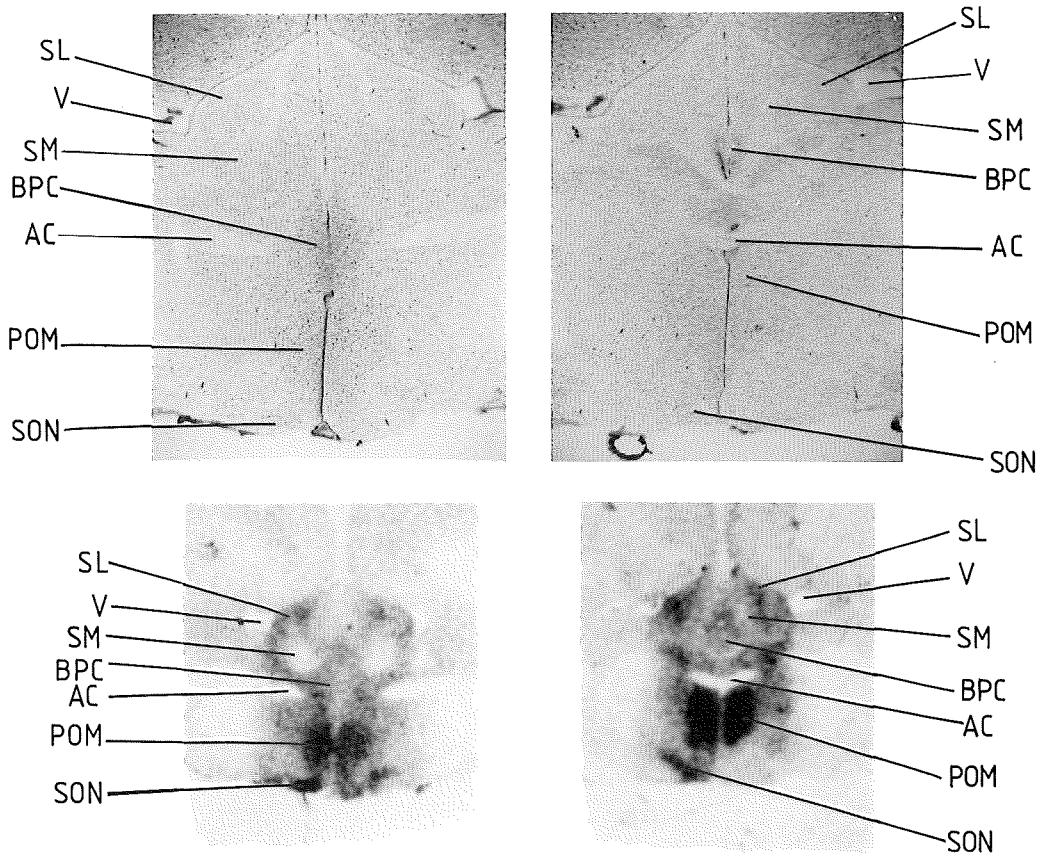


Fig. 5. Comparison of the sexually dimorphic POM nucleus as shown by thionin staining (upper panels) and by PAC binding (lower panels) in adjacent sections selected from a rostral (left panel) and a caudal (right panel) portion of the nucleus.

dorsalis (Mld) forms the torus semicircularis (see Fig. 6). We determined the position of a given binding measurement in ICo by relating it to the size of the adjacent Mld which contains almost no

$[^3\text{H}]\text{PAC}$ binding and whose area increases caudally. Therefore, all values of $[^3\text{H}]\text{PAC}$ binding in the lateral ICo were partitioned according to whether they were associated with an Mld that is smaller

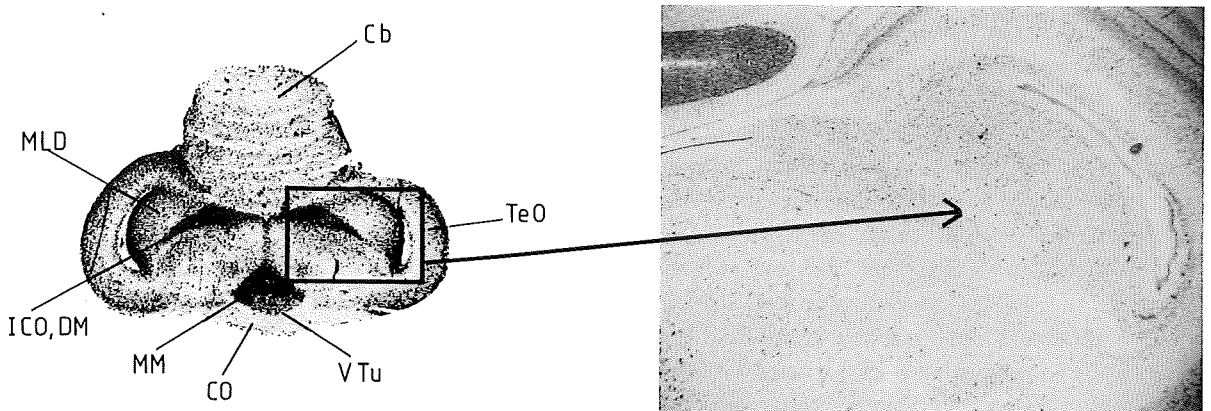


Fig. 6. Pattern of $[^3\text{H}]\text{PAC}$ binding in the intercollicular nucleus (ICo) and the nucleus mesencephalicus lateralis pars dorsalis (Mld) as compared with an adjacent thionin-stained section. Sections shown are from females.

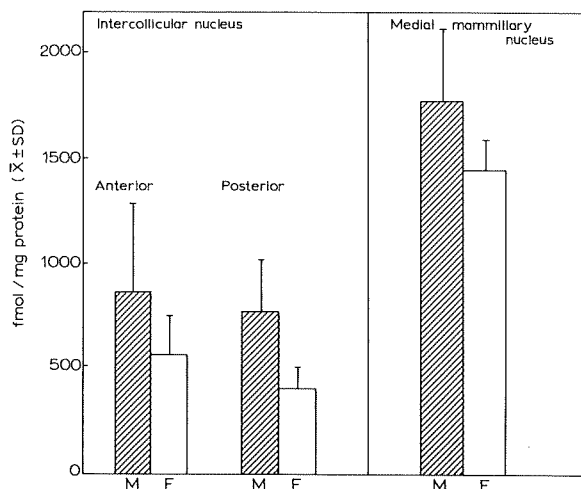


Fig. 7. Histograms illustrating the sex difference in density of [3 H]PAC binding in the rostral and caudal portions of the ICo (left panel). Relative position of the ICo was standardized in males and females by reference to the size of the non-dimorphic Mld (see text for details and statistical analyses). The right panel shows the sex difference in [3 H]PAC binding in the medial mammillary nucleus that was previously shown in Fig. 4. Data are expressed as fmol/mg protein as described in Materials and Methods.

(anterior level) or larger (posterior level) to the mean Mld size. Mld size was determined on the autoradiograms using the DUMAS. This analysis revealed a significant effect of sex ($F = 5.42$, $P < 0.031$) but not of position ($F = 0.78$, N.S.) nor of their interaction ($F = 0.91$, N.S.). As shown in Fig. 7, levels of α_2 -receptors were higher in males than in females at both the anterior and the posterior part of ICo. The rostro-caudal change in [3 H]PAC binding is thus not responsible for the observed sex difference.

DISCUSSION

In the studies reported here, quantitative autoradiography was used to investigate the distribution and density of α_2 -receptors in quail brain. Our focus centered principally on brain areas that have been implicated in reproductive processes and on differences between sexes. In preliminary studies, [3 H]-PAC, the ligand used to label α_2 receptors, was found to bind to sites in quail brain with characteristics similar to those previously described for mammals. In particular, [3 H]PAC was found to bind with high affinity and saturability in autoradiographic

studies with selected brain nuclei (Inf, DMA, AV, and VMN) and in experiments in which bound [3 H]PAC was determined by scintillation counting (sections taken from minced and molded hypothalami were used for the latter experiments to decrease inter-section variability and to circumvent the time delay associated with the generation of autoradiograms). By validating the assay with the use of autoradiography in 4 diverse brain nuclei, we have established that differences in apparent receptor density detected throughout the brain are the result of differences in the number of binding sites rather than differences in receptor affinity. In addition, agonists and antagonists competed for [3 H]PAC binding sites with an order of potency indicative of α_2 -receptors. It is interesting to note that as in mammals such as guinea pigs³⁴ and rats⁵⁶, epinephrine showed a higher affinity than NE for the α_2 -adrenergic receptor. In birds, this has potential biological significance because of the substantial amounts of epinephrine that have been detected in the avian brain^{28,30} in contrast to mammalian forms⁴⁰.

In general, the areas of the quail brain containing high levels of α_2 -receptors are similar to those of mammals. Absolute levels of binding are in the same order of magnitude as those reported for guinea pigs²⁵ and rats⁵⁶. The maximum level of binding observed in some nuclei is, however, 4–5 times higher than maximum levels reported in mammals. This could be related to the fact that norepinephrine brain concentrations are, in general, higher (at least 2 to 3 fold) in quail^{30,38,39} than in rats^{44,45} and guinea pigs²⁶. In the quail, as in the rat and the guinea pig, some of the highest concentrations of α_2 -receptors occur in hypothalamic structures, parts of the limbic system, and in preoptic nuclei. Major contrasts do exist, however. For example, the highest level in the rat was in the CA1 regions of the hippocampus⁵⁶. Since the hippocampus is poorly defined in birds, it is not known exactly what the homologue of that region is in birds; however, in areas classically identified as hippocampus α_2 -binding is low relative to other brain areas (see Fig. 3 and 4).

Sex differences in [3 H]PAC binding were detected in two brain nuclei, the medial mammillary nuclei and the ICo. The differences reported here are of a similar magnitude to that reported in guinea pig

studies that analyzed sex differences and steroid effects on α_2 -receptor density^{25,27}. It is possible that the sex differences may have occurred by chance as a result of repeated sampling in different brain areas. However, this seems unlikely in view of the fact that the observed differences were restricted to steroid-sensitive areas implicated in the control of reproductive processes. These differences could be the result of hormonal events early in life that irreversibly differentiate the sexes (i.e. organizational effects). Alternatively, the differences could be maintained by sex differences in circulating levels of hormone and disappear if the hormones are removed, similar to what appears to be the case for the dimorphism in size in the POM⁴¹. If these differences are mediated by steroid hormones either acting early in life or in adulthood, it is possible that they could have been brought about by steroid induced changes in neuronal growth¹⁸. Changes in tissue density affect autoradiographic efficiency, and differences in optical density in these areas might then be due to sex differences in tissue density rather than to differences in [³H]PAC binding. This possibility cannot, at present, be totally excluded. However, we have not detected sex differences in binding in these areas for muscarinic cholinergic receptors as labelled with [³H]*n*-methyl scopolamine²⁰ so it seems likely that the differences detected result from genuine differences in α_2 -receptor binding.

Both areas showing sex differences in binding are involved in reproductive processes, which suggests that the differences in α_2 -binding may have physiological relevance. [³H]PAC binding was significantly higher in females than in males. The medial mammillary nuclei are located in the middle of the infundibular region which is a critical site for the control of gonadotrophin secretion¹⁹. This region contains high concentrations of norepinephrine and epinephrine³⁰ and catecholamine containing fibers have been identified by histofluorescence⁵³. MM is also adjacent to the paraventricular organ (PVO³²). The PVO has access to the cerebro-spinal fluid (CSF) but apparently not to the systemic circulation⁶¹. MM could thus be a major site for the interaction of GnRH with substances in the CSF. The presence of a sexual dimorphism in α_2 -adrenergic receptors in this nucleus may be related to the sexual dimorphism in plasma gonadotropins; α -

adrenergic receptors are higher in males than in females and in quail LH levels are in general higher in males^{48,57}.

Similarly, the sex difference in [³H]PAC binding observed in the lateral ICo might be related to the sexual dimorphism in quail vocalizations. Male and female quail have a number of sex-specific vocalizations²³ of which crowing is the best characterized. Crowing occurs only in males. Some reports showed that even after gonadectomy and treatment with testosterone, crowing frequency and crowing quality are still different in males and females^{1,5}, although other studies failed to find such a difference (e.g. 6,47). ICo has been identified as a critical nucleus for the production of vocal behavior in a variety of avian species including quail and related forms^{15,42,52}. The existence of a sexual dimorphism in α_2 receptors in this area in the quail brain is suggestive of a possible neurochemical factor underlying the sexually dimorphic behavior. Thus, sexually dimorphic vocal behavior in the quail may be mediated by neurochemical differences without the concomitant morphological dimorphisms that have been described in the system controlling vocal production in songbirds^{3,31,35}. The autoradiographic analysis suggested that the sex difference in receptor concentration is located primarily in the lateral part of ICo. The major proportion of the projections to and from ICo in songbirds that are relevant to vocal control are localized to the dorsomedial subregion of ICo^{22,37}. However, fibers from a vocal control area dorsal to ICo, the robust nucleus of the archistriatum, appear to innervate both the lateral and medial portions of ICo³⁶, and electrical stimulation in both portions elicits vocalizations¹⁴, suggesting that the entire nucleus is involved in vocal behavior.

The pattern of [³H]PAC binding provides an independent biochemical marker of the sexually dimorphic POM area^{41,58} within the preoptic area. Several lines of evidence suggest that this area plays a key role in the control of copulatory behavior in quail. It contains high concentrations of estradiol receptors as demonstrated by autoradiographic⁵⁹ and immunohistochemical⁸ techniques. Furthermore, stereotaxic implants of testosterone in or near POM activate sexual behavior in castrated male quail^{9,60}. Aromatization of testosterone into estradiol appears to be a critical step in the production of

this behavioral effect. The steroid metabolizing enzyme aromatase is particularly high in the POM⁵⁰ and is sexually differentiated. Enzyme activity in the preoptic area is higher in males than in females and this difference persists even when birds are gonadectomized and treated with similar amounts of testosterone⁴⁹. This nucleus is also located in the POM which was shown to contain sex differences in the concentrations of norepinephrine: females have higher concentrations than males³⁹. In that norepinephrine seems to play an inhibitory role in the control of reproductive behavior in birds, the POM is a possible site of integration of steroidal and catecholaminergic influences on behavior. In particular, the sexual dimorphism in copulatory behavior (testosterone activates copulation in castrated males but not in ovariectomized females^{2,5,47}), could result from a sexual difference in adrenergic inhibition of behavior occurring in the POM. The signal from such a differential inhibition is present (higher concentration of norepinephrine) and adrenergic receptors are also present. However, no sexual difference in receptor density could be detected here.

Examples of precise neurochemical labeling of dimorphic structures in the preoptic area are relatively rare. Commins and Yahr¹⁶ have used acetylcholinesterase staining to label the sexually dimorphic area (SDA) of the preoptic area of the gerbil. This does not identify the entire region however.

ABBREVIATIONS in the figures

Av	archistriatum, pars ventralis
BPC	bed nucleus pallial commissure
CA	commissura anterior
Cb	cerebellum
CDL	cortex dorsolateralis
CO	chiasma opticum
DMA	nucleus dorsomedialis anterior thalami
FPL	fasciculus prosencephali lateralis (= lateral forebrain bundle)
HA	hyperstriatum accessorium
Hp	hippocampus
HV	hyperstriatum ventrale
ICO	nucleus intercollicularis

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The most dimorphic portion of the SDA, the pars compacta, is in fact totally devoid of positive staining. Other neurochemical markers have been found to be sexually dimorphic in and around the SDA in rats (opiate receptor²⁴; serotonergic fibers⁵⁴). However, neither of these markers corresponds exactly with this dimorphic region. The labeling of a sexually dimorphic nucleus in the preoptic region with a specific neurotransmitter receptor has to our knowledge not previously been described. The close correspondence between the nucleus and α_2 -receptors provides insight into the functional characteristics of the nucleus. It suggests an important interaction between steroids and the adrenergic system in the control of sexually dimorphic behavior.

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Mld	nucleus mesencephalicus lateralis, pars dorsalis
MM	nucleus mamillaris medialis
PD	nucleus preopticus dorsolateralis
POM	preoptic medial nucleus
QF	tractus quintofrontalis
Rt	nucleus rotundus
SL	septum lateralis
SM	septum medialis
SON	nucleus supraopticus
TSM	tractus septomesencephalicus
TeO	tectum opticum
V	ventricle
VMN	nucleus ventromedialis hypothalami
VTu	ventral tube

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