Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 4766–4770, June 1989
Neurobiology

Estrogen differentially regulates neuropeptide gene expression in a sexually dimorphic olfactory pathway

(cholecystokinin/*in situ* hybridization/medial amygdala/substance P/tachykinin)

R. B. SIMERLY*, B. J. YOUNG, M. A. CAPOZZA, AND L. W. SWANSON

Howard Hughes Medical Institute and Salk Institute for Biological Studies, La Jolla, CA 92037

Communicated by C. H. Sawyer, March 20, 1989 (received for review December 30, 1988)

ABSTRACT **The posterodorsal part of the medial nucleus of the amygdala (MeAp) receives its major sensory input from the accessory olfactory bulb and projects massively to the medial preoptic nucleus and other sexually dimorphic hypothalamic nuclei thought to play key roles in mediating steroid-sensitive reproductive functions. A combined axonal transport/double-immunohistochemical method was used to show that at least one-quarter of the cholecystokinin-immunoreactive cells in the MeAp cocontain substance P and that a substantial proportion of these cells project to the medial preoptic nucleus. *In situ* hybridization histochemistry was then used to demonstrate that estrogen regulates the expression of preprocholecystokinin in these cells at the mRNA level in male and female rats. In contrast, levels of preprotachykinin mRNA within the MeAp do not appear to be sensitive to acute changes in circulating gonadal steroids in either sex. Although posttranscriptional regulation of mRNA stability may contribute to the observed effects, it appears likely that estrogen stimulates preprocholecystokinin expression within the MeAp by selectively inducing transcription of the corresponding gene, thereby altering the relative amounts of cholecystokinin and substance P coexpressed within individual neurons of the MeAp that project to the hypothalamus.**

Olfactory cues exert profound effects on reproductive behavior and gonadotropin secretion (1–3), and much of this sensory information appears to originate in the vomeronasal organ, which provides primary afferent fibers to the accessory olfactory bulb (AOB). Scalia and Winans (4) first described in detail the central projections of the rat AOB, which relays olfactory information from the vomeronasal organ to five specific regions: the posterior cortical nucleus of the amygdala, bed nucleus of the accessory olfactory tract, anterior and posterior parts of the medial nucleus of the amygdala, and encapsulated part of the bed nucleus of the stria terminalis (BSTe; see ref. 5). The AOB, posterodorsal part of the medial nucleus of the amygdala (MeAp), and BSTe are sexually dimorphic (6) and appear to relay olfactory information to parts of the hypothalamus known to be involved in reproductive function. We describe here combined axonal transport/immunohistochemical experiments demonstrating that the neuroactive peptides cholecystokinin (CCK) and substance P (SP), which are thought to participate in mediating reproductive events (7–11), are synthesized by cells along this pathway.

Gonadal steroid hormones appear to influence mature reproductive function by regulating cellular and molecular events associated with neurotransmission in specific neural pathways (12). Thus, gonadal steroids may alter sensory influences on reproductive neuroendocrine responses (3) by modulating neuropeptide gene expression within cells along olfactory pathways. Previous immunohistochemical studies indicate that estrogen regulates levels of CCK immunoreactivity (CCKir) in neurons of the MeAp, BSTe, and the central part of the medial preoptic nucleus (MPN; see ref. 5). In adult male rats, gonadectomy profoundly decreases the number of CCKir neurons within each area, and this effect can be reversed by testosterone replacement (5), as well as by treatment with estrogen or diethylstilbestrol (DES, a nonsteroidal estrogenic compound), but not by the androgen-agonist fluoxymesterone (unpublished data). Therefore, it is likely that estrogen, rather than testosterone, is responsible for the regulation of CCK peptide levels in male rats, presumably through intraneuronal aromatization of testosterone to estrogen (13). In females, gonadectomy also decreases CCKir levels in these three nuclei, and again the effect is reversed by estrogen treatment (14). Furthermore, the number of CCKir neurons was found to vary over the estrous cycle from a minimum at diestrus to a maximum at proestrus, following natural variations in circulating levels of gonadal steroids (14). We report here *in situ* hybridization evidence supporting the view that CCK expression within neurons of this sexually differentiated olfactory pathway is regulated by estrogen in a physiologically relevant way in both male and female rats at the mRNA level.

METHODS

Axonal Transport–Immunohistochemical Experiments. The projections of the MeAp and BSTe were traced by placing iontophoretic injections of *Phaseolus vulgaris* leucoagglutinin (PHA-L) into each cell group and immunohistochemically localizing axons containing the anterogradely transported lectin according to methods described in detail elsewhere (15, 16). CCK and SP were colocalized within individual neurons in colchicine-treated male and female rats using a double-immunohistochemical method (see ref. 17 for details). The rabbit antiserum to CCK (Immuno Nuclear, Stillwater, MN) was combined in solution with a mouse monoclonal antibody to SP (Sera-Lab, Crawley Down, Sussex, U.K.); primary antibodies were localized with lisamine-rhodamine-, and fluorescein-isothiocyanate-conjugated goat anti-rabbit and mouse secondary antibodies, respectively. Appropriate control experiments (see ref. 17) demonstrated the absence of either primary or secondary cross-reactivity. Neurons in the MeAp containing CCKir and SP immunoreactivity (SPir)

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: DES, diethylstilbesterol; AOB, accessory olfactory bulb; AVPv, anteroventral periventricular nucleus; BSTe, encapsulated part of the bed nucleus of the stria terminalis; CCK, cholecystokinin; CCKir, CCK immunoreactivity; MeAp, posterodorsal part of the medial nucleus of the amygdala; MPN, medial preoptic nucleus; pCCK, preprocholecystokinin; pT, preprotachykinin; SP, substance P; SPir, SP immunoreactivity; PHA-L, *Phaseolus vulgaris* leucoagglutinin.

*To whom reprint requests should be addressed at: Neural Systems Laboratory, Salk Institute, P.O. Box 85800, San Diego, CA 92138-9216.

were shown to project to the MPN with iontophoretic injections of the retrograde tracer fluorogold (2% in saline; see ref. 18) into the MPN. Two weeks later these animals received colchicine injections and were perfused after an additional 3 days (see ref. 19). Sections through the MeAp were processed for CCK and SP double-immunohistochemistry as described above.

Experimental Animals. Adult male and female rats were used for the tract-tracing/immunohistochemical experiments. Male animals were intact; females were ovariectomized on day 60 of life and immediately implanted with pellets of estradiol-17β designed to deliver a constant dose of steroid for at least 3 weeks (Innovative Research of America). Animals for the *in situ* hybridization experiments were prepared as described below and processed as three separate experiments. Animals in each experiment were sacrificed and processed in parallel.

Experiment 1. Gonadectomized adult male Sprague–Dawley rats received pellets of DES (0.1 mg/pellet) 14 days after gonadectomy or were left untreated. Both groups of animals were perfused 28 days after gonadectomy. *Experiment 2.* Adult female Sprague–Dawley rats (50–65 days old) were used in this experiment. Vaginal smears were monitored daily for at least two consecutive 4-day cycles; animals exhibiting vaginal smears characteristic of proestrous or metestrous animals were perfused. *Experiment 3.* Normally cycling female Sprague–Dawley rats were ovariectomized on day 60 of life. Half of these animals received pellets containing estradiol-17β (0.5 mg) designed to deliver levels of circulating estradiol within the physiological range as confirmed by RIA. The remaining animals received control pellets, and both groups of animals were perfused 7 days later.

***In Situ* Hybridization.** The protocol essentially follows that of Angerer *et al.* (20) and is described in detail elsewhere (21). Briefly, SP6 and T7 polymerases were used to synthesize asymmetric RNA probes complementary to preprocholecystokinin (pCCK) (22) and preprotachykinin (pT) (23) mRNA, respectively, from full-length cDNA inserts. The pT insert (560 base pairs) contained in pGEM1 (Promega) was supplied by J. E. Krause (Washington University, Saint Louis), has sequences complementary to α-, β-, and γ-pT mRNA, and hybridizes to all three mRNA species (24). The pCCK insert (527 base pairs) was supplied by J. E. Dixon (Purdue University, West Lafayette, IN) and subcloned into the *Hin*dIII and *Eco*RI sites of pGEM4 (Lofstrand Laboratories, Gaithersburg, MD). The radiolabeled cRNA probes were diluted to 1.5×10^7 dpm/ml with hybridization buffer (see ref. 21). Sections were incubated in this hybridization solution for 20 hr at 58°C, conditions that allow saturation of hybridizable pCCK and pT mRNA as determined by RNA over time analysis (see ref. 20). After hybridization the slides were rinsed in 4× SSC (1× SSC is 0.15 M sodium chloride/0.015 M sodium citrate) before RNase digestion (20 μg/ml for 30 min at 37°C) and then rinsed in decreasing concentrations of SSC to a final stringency of 0.1× SSC at 55°C for 30 min. After dehydration in alcohol the sections were dipped in NTB-2 (Kodak) liquid emulsion. The autoradiograms were developed 14 days later, and the sections were counterstained through the emulsion with thionin.

Quantification. pCCK and pT mRNA-containing cells were counted at a magnification of 400× and were considered labeled when the density of silver grains over thionin-stained cells exceeded 5× background levels. This rather conservative labeling criterion was chosen to eliminate the possibility of including ambiguously labeled cells in the cell counts. Corrections for double-counting errors were made according to Abercrombie (25).

RESULTS

Accessory Olfactory Pathway. In the first series of experiments, we examined the organization of neural pathways leading from the MeAp and BSTe to the hypothalamus. Injections of PHA-L centered in the MeAp (but not the anterior part of the medial nucleus of the amygdala) labeldense plexuses of axons and terminal boutons in four sexually dimorphic cell groups involved in reproductive function (Fig. 1): the central and medial parts of the medial preoptic nucleus (Fig. 2 *A* and *B*), which appear to play a critical role in male copulatory behavior (27, 28); the ventrolateral part of the ventromedial hypothalamic nucleus, which appears to play a critical role in female copulatory behavior (29); and the anteroventral periventricular nucleus, a preoptic nucleus thought to play a critical role in the cyclic release of gonadotropin (30), and the BSTe (Fig. 2*B*). Similarly, injections confined to the BSTe labeled terminal fields in the same parts of the MPN, ventromedial nucleus, and anteroventral periventricular nucleus. These results suggest that pheromonal information may be transmitted from the AOB to the medial preoptic, ventromedial, and anteroventral periventricular nuclei along a series of subdivision-specific connections between sexually dimorphic cell groups in the rat forebrain.

CCK and SP in the AOB Pathway. Within the medial nucleus of the amygdala and bed nucleus of the stria terminalis, CCKir and SPir neurons are localized almost entirely to the BSTe and MeAp. By applying a double-immunostaining method we found that a substantial number of CCKir neurons within the MeAp also contain SPir. Furthermore, we demonstrated that a population of neurons containing both CCKir and SPir project to the MPN. For this, injections of the retrograde fluorescent tracer, fluorogold, were placed in the MPN of male and female rats, and tissue sections were processed for double immunofluorescence labeling (Fig. 2 *C–F*). Injections were centered in the central part of the MPN, and the effective injection site did not appear to spread significantly beyond the borders of the MPN. Within the amygdala, retrogradely labeled neurons were largely limited to the MeAp, and many of these cells also contained CCKir, SPir, or were triply labeled. Cell counts in this material indicate that in both sexes at least a quarter of the CCKir neurons contain SPir and that at least a quarter of the neurons that contain both peptides project to the MPN. However, these semi-quantitative estimates probably represent minimum values because all CCKir and SPir cells may not have been detected, and all of the MeAp cells that project to the MPN were probably not retrogradely labeled.

Estrogen Regulates CCK but Not SP Expression. Finally, hybridization histochemistry was used to determine whether changes in circulating gonadal steroids alter cellular levels of

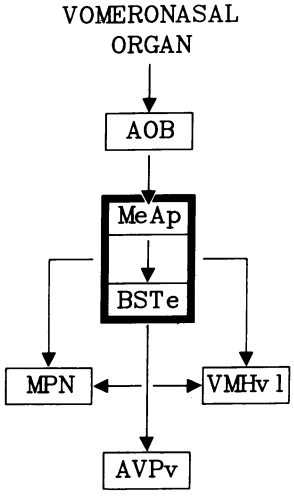

FIG. 1. Diagram summarizing key aspects of forebrain circuitry thought to integrate olfactory and gonadal steroid feedback information influencing reproductive function. The MPN has been shown to be involved in both male and female sexual behavior, the ventrolateral part of the ventromedial hypothalamic nucleus (VMHvl) plays a key role in mediating female sexual behavior, and the AVPv is a nodal point in the neural circuitry regulating gonadotropin secretion.

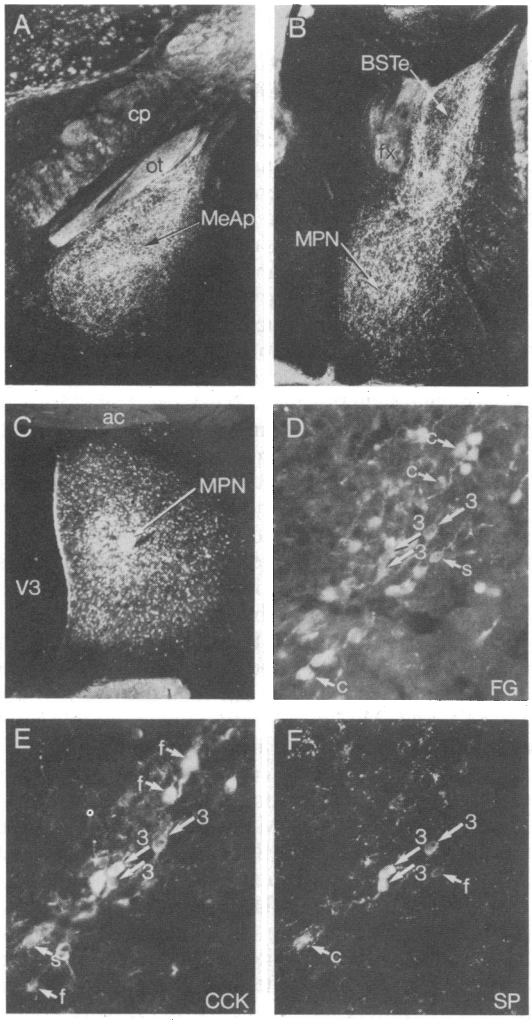

FIG. 2. CCK and SP are colocalized within neurons of the MeAp that project to the MPN. (*A* and *B*) An iontophoretic injection of PHA-L was made into the MeAp, and axons containing the transported lectin were labeled immunohistochemically. Dense plexuses of labeled axons and terminal boutons are labeled in the medial MPN and outer part of the BSTe. (*A* and *B*, ×25.) (*C*) An iontophoretic injection of the retrograde tracer fluorogold was made into the MPN of an estrogen-treated, ovariectomized female rat. (×20.) (*D*) Retrogradely labeled neurons in the MeAp contained CCKir (arrows from c), SPir (arrow from s), or were triply labeled (arrows from 3). (×140.) (*E* and *F*) The same field as in *D* to illustrate CCKir neurons (*E*) that also contain fluorogold (arrows from f), or SPir (arrows from s) and SPir neurons (*F*) that also contain fluorogold (arrow from f), or CCKir (arrows from c). Triply labeled neurons are indicated (arrows from 3). (×140.)

pCCK and pT mRNAs, which encode the preprohormones for CCK and SP, respectively. Gonadectomy of male rats caused a dramatic reduction in the amount of pCCK mRNA hybridization over neurons in the MeAp (Fig. 3*A*), and treatment of 14-day castrates for an additional 14 days with DES reversed this effect (Fig. 3*B*). In contrast, no discernible difference in pT hybridization was found between gonadectomized and DES-treated male animals (Fig. 3 *C* and *D*). Similarly, treatment of ovariectomized female rats with estradiol prevented the decline in pCCK mRNA labeling over neurons in the MeAp seen after ovariectomy alone (Fig. 4 *A* and *B*). Again, gonadectomy had no measurable influence on pT hybridization in female animals.

Further experiments suggested that this estrogenic influence on pCCK mRNA levels in the MeAp can occur under physiological conditions in the female (Fig. 4 *C* and *D*). Thus, pCCK mRNA levels were low during metestrus (when circulating estradiol levels are lowest) and were significantly higher during proestrus (when estradiol levels are considerably higher). No significant differences in pT hybridization were found between these two phases of the estrous cycle. These apparent changes in pCCK expression were substantiated by counting the number of pCCK- and pT-labeled neurons in the MeAp that exceeded a labeling criterion of five times background levels (Fig. 5).

Variations in the number of pCCK-hybridized neurons in the MeAp appear to be due to changes in the level of mRNA within individual cells. In general, the density of silver grains over labeled neurons in the MeAp of gonadectomized male and female rats was considerably lower than that seen over neurons in steroid-treated animals (Fig. 4 *E* and *F*). As might be expected, a range of labeling intensities was seen for all groups, but the examples shown in Fig. 4 were typical. Preliminary estimates of grain densities over individual cells suggest that estrogen treatment may cause as much as a 3-fold increase in the number of silver grains per cell compared with untreated control animals. Finally, it is important to point out that similar changes in pCCK hybridization were seen in the central part of the MPN and BSTe (unpublished data) in accordance with our earlier immunohistochemical evidence (5, 14).

DISCUSSION

The MPN, BSTe, and MeAp contain very high densities of gonadal steroid-concentrating neurons (31, 32), and each cell group has been implicated in the control of both copulatory behavior and the secretion of luteinizing hormone (see ref. 5). Therefore, it appears likely that these sexually dimorphic cell groups participate in mediating the feedback effects of gonadal steroids on reproductive function in the adult. Furthermore, combined retrograde tracer/autoradiographic experiments have shown that many neurons in the MeAp and BSTe that project to the MPN concentrate circulating gonadal steroids (32), suggesting that the direct effects of gonadal steroids on cells in the MPN may be complemented by steroid-induced neural activity in cells of the BSTe and MeAp (34), which, in turn, may be relayed to the MPN by means of direct axonal projections from both regions. The involvement of this pathway in relaying olfactory information from the AOB to the hypothalamus suggests that these cell groups may form part of a sexually dimorphic, gonadal steroid-sensitive neural circuit involved in the integration of humoral and sensory information related to the control of reproductive behavior and physiology. The cellular mechanisms underlying this neurohumoral integration are unclear, but our results suggest that one contributing factor may be the regulation of neuropeptide gene expression by gonadal steroids within cells along this sensory pathway.

The interpretation of previous immunohistochemical experiments addressing the possible regulation of CCK within these cells by estrogen is complicated by the necessary use of colchicine pretreatment to visualize maximal numbers of immunoreactive cell bodies. In addition, these experiments do not address whether estrogen regulates CCK biosynthesis or decreases degradation of the peptide. Furthermore, if estrogen does increase CCK biosynthesis, it is important to establish the mechanism involved. The results of our *in situ* hybridization experiments begin to address these questions and indicate that in both male and female rats estrogen alters CCK levels within cells of the central MPN, BSTe, and MeAp by regulating levels of pCCK mRNA at either the transcriptional or posttranscriptional level. Although the possibility that posttranscriptional regulation of pCCK mRNA stability may contribute to the observed effects, given what is currently known about the molecular mechanisms by

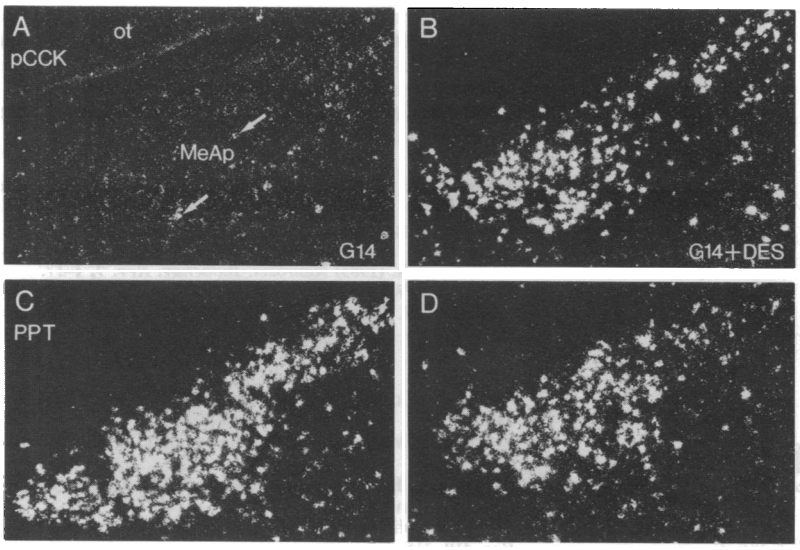

FIG. 3. DES alters levels of pCCK, but not pT, mRNA within neurons of the MeAp in male rats. (*A* and *B*) Dark-field photomicrographs comparing density of pCCK mRNA-containing neurons within the MeAp of a male rat 28 days after castration (*A*), with that of a 14-day castrate (G14) that was treated with DES for 14 days (G14 + DES; *B*). (*C* and *D*) Dark-field photomicrographs to show the relative densities of pT mRNA-containing neurons in sections adjacent to those shown in *A* and *B*, respectively.

which steroid hormones affect gene expression (see refs. 35, 37), it appears likely that estrogen stimulates pCCK expression within the MeAp by inducing transcription of the pCCK gene. The resolution of this question will require further study, and the possibility that neural inputs to CCK cells may alter pCCK expression should also be investigated. Finally, it appears that the regulation of pCCK expression may be gene specific because we did not observe comparable changes in levels of cellular pT mRNA in any of the experimental animals despite the fact that SP is coexpressed within a subpopulation of CCKir cells. Whether or not estrogen exerts more subtle, long-term regulatory effects on SP expression remains to be determined.

The activational regulation of CCK expression by circulating gonadal steroids described here is particularly interesting because it occurs within three groups of neurons that are part of a sexually dimorphic pathway relaying olfactory influences from the vomeronasal organ to hypothalamic and limbic regions known to be critical for the normal expression of reproductive behavior and gonadotropin release. By focusing on neurotransmitter-specific cell types within this specific sensory pathway it may therefore be possible to clarify cellular mechanisms underlying gonadal steroid regulation of olfactory influences on reproduction. The results of our immunohistochemical and hybridization histochemical studies suggest that one of the ways in which gonadal steroids alter the functional output of neurons along this olfactory pathway is by differentially regulating neuropeptide gene expression.

Such differential regulatory influences imply that estrogen changes the ratio of neuropeptides in certain neurons, raising the intriguing possibility of "chemical switching" in the underlying neural circuitry (38). The central points of this model are that the colocalization of neurotransmitters implies that a mixture of both is released from all axon terminals of

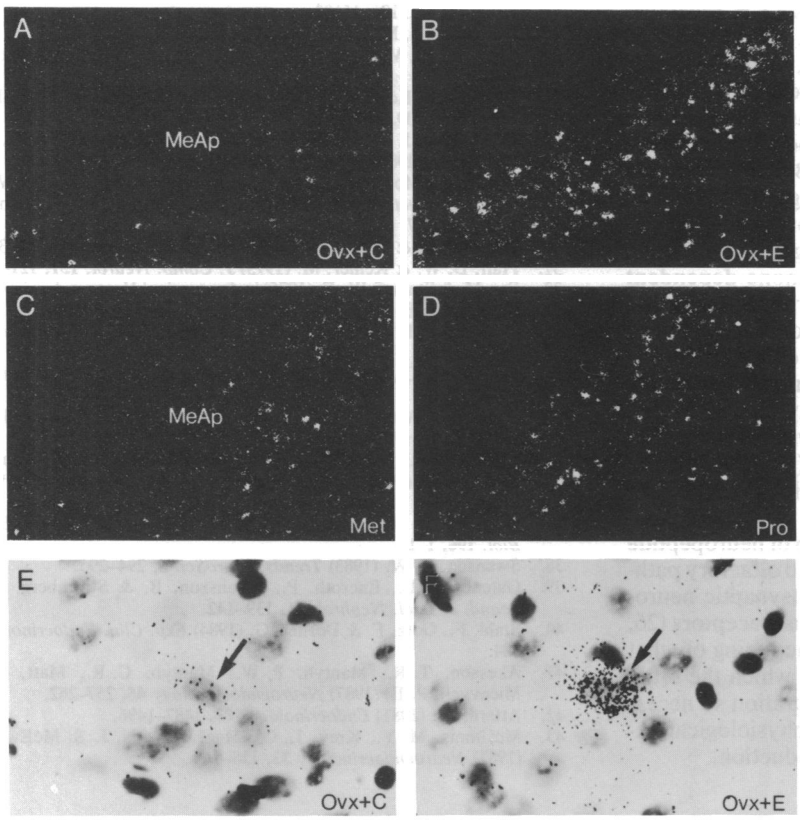

FIG. 4. Estrogen regulates pCCK mRNA levels within neurons of the MeAp in female rats. (*A–D*) Dark-field photomicrographs comparing the densities of pCCK mRNA-containing neurons in the MeAp of ovariectomized (Ovx) female rats implanted with control (Ovx + C; *A*), and estradiol (Ovx + E; *B*) pellets. (×50.) Similar changes occur over the estrous cycle as shown by the different densities of pCCK mRNA-containing neurons found in metestrous (Met; *C*) and proestrous (Pro; *D*) female rats. (×50.) (*E* and *F*) Characteristic examples of cellular labeling found for pCCK mRNA-containing neurons within the MeAp of control (*E*) and estrogen-treated females. (*F*; ×530.)

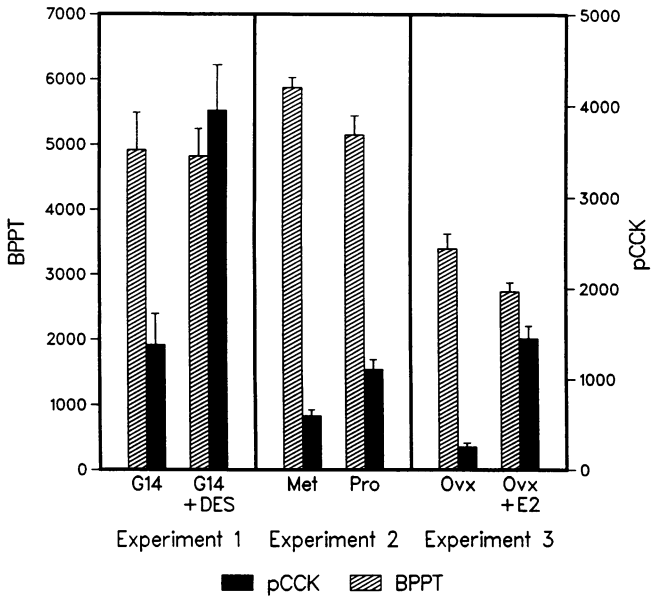

FIG. 5. Semiquantitative examination of the effects of gonadal steroids on pCCK and pT mRNA-hybridized neurons. Mean number (±SEM) of pCCK mRNA neurons within MeAp in experiment 1—gonadectomized control (G14) and DES-treated (G14 + DES) male rats; experiment 2—metestrous (Met) and proestrous (Pro) female rats; experiment 3—females ovariectomized and implanted with control pellets (Ovx) or estradiol (Ovx + E2). Significant ($P \leq 0.05$; two-tailed Mann–Whitney U test) differences were found in the number of pCCK mRNA cells found within the MeAp in all three experiments ($n = 4$ for each group).

a neuron (Dale's principle) and that the functional impact of this release depends on the ratio of transmitters as well as on the complement of postsynaptic receptors at each terminal. Therefore, if receptor levels remain constant and levels of peptide release remain proportional to axonal firing rate, the influence of a neuron in the MeAp expressing both CCK and SP on cell types that preferentially express CCK receptors would be relatively low during metestrus, when levels of circulating estrogen are low. In contrast, when estrogen levels increase during the afternoon of diestrus and early proestrus, intracellular levels of CCK increase, which should dramatically increase the activity of those postsynaptic cells that are preferentially sensitive to CCK. Because estrogen does not appear to cause comparable changes in SP levels, cellular responses mediated by SP receptors would be unchanged. Thus, within such a circuit, changes in circulating levels of estrogen would trigger a hormone-dependent "chemical switching" of sensory and other neural information transmitted by this cell. It remains to be established whether a combination of postsynaptic cell types in the circuitry shown in Fig. 1 contributes to estrogen-dependent reproductive responses that occur normally during the estrous cycle, or that occur under certain physiological conditions such as chronic stress in the male (39, 40), but the present findings suggest that these possibilities should be explored.

Superimposed on the estrogenic regulation of neuropeptide gene expression within sexually differentiated olfactory pathways is the possible regulation of both postsynaptic neurotransmitter receptors (41) and steroid hormone receptors (26, 42, 43) by estrogen, adding further to the fine tuning of what must be an acutely sensitive mechanism by which the brain accomplishes the endocrine and neural integration so necessary for the coordinated behavioral and physiological responses that characterize mammalian reproduction.

We thank Kris Trulock for photographic assistance and Dave Warren for secretarial help. We are also grateful to Drs. Jack Dixon and James Krause for providing pCCK and pT cDNAs, as well as helpful comments on an earlier version of the manuscript. We also thank Drs. Stanley Watson and Jeffrey Arriza for valuable suggestions and discussions.

1. Johns, M. A. (1980) in *Chemical Signals, Vertebrates and Aquatic Invertebrates*, eds. Muller-Schwarze, D. & Silverstein, R. M. (Plenum, New York), pp. 341–364.
2. Wysocki, C. J. (1979) *Neurosci. Biobehav. Rev.* **3**, 301–341.
3. Gandelman, R. (1983) *Neurosci. Biobehav. Rev.* **7**, 1–17.
4. Scalia, F. & Winans, S. W. (1975) *J. Comp. Neurol.* **161**, 31–56.
5. Simerly, R. B. & Swanson, L. W. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2087–2091.
6. del Abril, A., Segovia, S. & Guillamón, A. (1987) *Dev. Brain Res.* **32**, 295–300.
7. Aronin, N., Coslovsky, R. & Leeman, S. E. (1986) *Annu. Rev. Physiol.* **48**, 537–549.
8. Babcock, A. M., Bloch, G. J. & Micevych, P. E. (1988) *Physiol. Behav.* **43**, 195–199.
9. Bloch, G. J., Babcock, A. M., Gorski, R. A. & Micevych, P. E. (1988) *Physiol. Behav.* **39**, 217–224.
10. Kimura, F., Mitsugi, N., Arita, J., Akema, T. & Yoshida, K. (1987) *Brain Res.* **410**, 315–322.
11. Elliott, P. J. & Iversen, S. D. (1986) *Brain Res.* **381**, 68–76.
12. McEwen, B. S. & Pfaff, D. W. (1985) *Trends NeuroSci.* **8**, 105–110.
13. Celotti, F., Massa, R. & Martini, L. (1979) in *Endocrinology*, ed. DeGroat, L. J. (Grune & Stratton, New York), pp. 41–53.
14. Oro, A. E., Simerly, R. B. & Swanson, L. W. (1988) *Neuroendocrinology* **47**, 225–235.
15. Gerfen, C. R. & Sawchenko, P. E. (1985) *Brain Res.* **343**, 144–150.
16. Simerly, R. B. & Swanson, L. W. (1988) *J. Comp. Neurol.* **270**, 209–242.
17. Sawchenko, P. E., Swanson, L. W., Grzanna, R., Howe, P. R. C., Bloom, S. R. & Polak, J. M. (1985) *J. Comp. Neurol.* **241**, 138–153.
18. Schmued, L. C. & Fallon, J. H. (1986) *Brain Res.* **377**, 147–154.
19. Simerly, R. B., McCall, L. D. & Watson, S. J. (1988) *J. Comp. Neurol.* **276**, 442–459.
20. Angerer, L. M., Stoler, M. H. & Angerer, R. C. (1981) in *In Situ Hybridization*, eds. Valentino, K. L., Eberwine, J. H. & Barchas, J. D. (Oxford Univ. Press, New York), pp. 42–70.
21. Arriza, J. L., Simerly, R. B., Swanson, L. W. & Evans, R. M. (1988) *Neuron* **1**, 887–900.
22. Deschenes, R. J., Haun, R. S., Funckes, C. L. & Dixon, J. E. (1985) *J. Biol. Chem.* **260**, 1280–1286.
23. Krause, J. E., Chirgwin, J. M., Carter, M. S., Xu, Z. S. & Hershey, A. D. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 881–885.
24. MacDonald, M. R., McCourt, D. W. & Krause, J. E. (1988) *J. Biol. Chem.* **263**, 15176–15183.
25. Abercrombie, M. (1946) *Anat. Rec.* **94**, 239–247.
26. Clark, C. R., MacLusky, N. J. & Naftolin, F. (1982) *J. Endocrinol.* **93**, 339–354.
27. Hansen, S., Köhler, C., Goldstein, M. & Steinbusch, H. V. M. (1982) *Brain Res.* **239**, 213–232.
28. Turkenburg, J. L., Swaab, D. F., Endert, E., Louwerse, A. L. & van de Poll, N. E. (1988) *Brain Res. Bull.* **21**, 215–224.
29. Pfaff, D. & Modianos, D. (1985) in *Handbook of Behavioral Neurobiology: Reproduction*, eds. Adler, N., Pfaff, D. & Goy, R. W. (Plenum, New York), Vol. 7, pp. 423–493.
30. Wiegand, S. & Terasawa, E. (1981) *Neuroendocrinology* **34**, 395–404.
31. Pfaff, D. W. & Keiner, M. (1973) *J. Comp. Neurol.* **151**, 121–158.
32. Sar, M. & Stumpf, W. E. (1975) in *Anatomical Neuroendocrinology*, eds. Stumpf, W. E. & Grant, L. D. (Karger, Basel), pp. 120–133.
33. Akesson, T. R., Simerly, R. B. & Micevych, P. E. (1988) *Brain Res.* **451**, 381–385.
34. Schiess, M. C., Joels, M. & Shinnick-Gallagher, P. (1988) *Brain Res.* **440**, 380–385.
35. Beato, M., Arnemann, J., Chalepakis, G., Slater, E. & Willmann, T. (1987) *J. Steroid Biochem.* **27**, 9–14.
36. Chambon, P., Dierich, A., Gaub, M.-P., Jakowlev, S., Jongstra, J,. Krust, A., LePennec, J.-P., Oudet, P. & Reudelhuber, T. (1984) *Rec. Prog. Horm. Res.* **40**, 1–42.
37. O'Malley, B. W., Schrader, W. T. & Tsai, M.-J. (1986) *Adv. Exp. Med. Biol.* **196**, 1–10.
38. Swanson, L. W. (1983) *Trends NeuroSci.* **6**, 294–295.
39. Gatenbeck, L., Eneroth, P., Johansson, B. & Stromberg, L. (1987) *Scand. J. Urol. Nephrol.* **21**, 139–142.
40. Stahl, F., Gotz, F. & Dörner, G. (1984) *Exp. Clin. Endocrinol.* **84**, 277–284.
41. Akesson, T. R., Mantyh, P. W., Mantyh, C. R., Matt, D. W. & Micevych, P. E. (1987) *Neuroendocrinology* **45**, 257–262.
42. Attardi, B. (1981) *Endocrinology* **108**, 1487–1496.
43. McGinnis, M. Y., Krey, L. C., MacLusky, N. J. & McEwen, B. S. (1981) *Neuroendocrinology* **33**, 158–165.