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Cholecystokinin and tyrosine hydroxylase messenger RNAs in neurons of rat mesencephalon: peptide/monoamine coexistence studies using in situ hybridization combined with immunocytochemistry

K. Seroogy[1,*], M. Schalling [1], S. Brené[1], Å. Dagerlind[1], S. Y. Chai[1], T. Hökfelt[1], H. Persson[2], M. Brownstein[3], R. Huan[4], J. Dixon[4], D. Filer[5], D. Schlessinger[5], and M. Goldstein[5]

Departments of [1]Histology and Neurobiology and [2]Molecular Neurobiology, Karolinska Institute, S–104 01 Stockholm, Sweden, [3]National Institute of Mental Health, Clinical Neuroscience Branch, Neuroendocrine Unit, Bethesda, MD 20205, USA, [4]Department of Biochemistry, Purdue University, West Lafayette, IN 47907, USA, [5]Department of Psychiatry, New York University Medical Center, New York, NY 10016, USA

Summary. The cellular localization of neurons expressing cholecystokinin (CCK) and tyrosine hydroxylase (TH) mRNAs was analysed in rat ventral mesencephalon using in situ hybridization techniques with both complementary DNA and synthetic oligonucleotide probes. Cell bodies distributed throughout the substantia nigra, ventral tegmental area, interfascicular nucleus, midline raphe nuclei, and central and ventral periaqueductal grey matter were found to contain CCK mRNA or TH mRNA as indicated by high densities of grains overlying the perikarya. The in situ hybridization technique was combined with immunocytochemistry on the same tissue section to localize the peptide or enzyme within its respective mRNA-containing somata. In addition, the presence of TH immunoreactivity was demonstrated within cell bodies labeled for CCK mRNA and immunostaining for CCK was detected within TH mRNA-containing neurons. In the medial geniculate nucleus a strong labeling for CCKmRNA was observed, in spite of the fact that so far no CCK-like immunoreactivity has been demonstrated in perikarya in this nucleus. The specificity of the probes was verified by RNA blot hybridization. These results confirm recent double-labeling immunocytochemical studies and further characterize the coexistence of CCK and TH at the level of their mRNAs as well as their post-translational products in a large population of mesencephalic dopamine neurons known to project to forebrain areas.

Key words: CCK – Dopamine – Messenger RNA – Complementary DNA – Oligonucleotides – Colocalization

* Present address: Department of Physiology, University of North Carolina, Chapel Hill, NC 27599, USA
Offprint requests to: T. Hökfelt (address see above)

Introduction

The ventral mesencephalon contains a dense population of dopaminergic neurons that are most concentrated in the substantia nigra and ventral tegmental area (Dahlström and Fuxe 1964; Lindvall and Björklund 1983). These neurons give rise to the ascending dopaminergic mesotelencephalic system which has been shown to play an important role in the modulation of extrapyramidal motor behavior (e.g. Fink and Smith 1980; Mason 1984) and has been implicated in the dysfunctions of Parkinson's disease (Hornykiewicz 1980; Javoy-Agid et al. 1984), schizophrenia (Randrup and Munkrad 1972; Snyder et al. 1974; Matthysse and Kety 1975; Meltzer and Stahl 1976), and Huntington's disease (Bruyn 1968; Spokes 1980). The peptide cholecystokinin (CCK) (Mutt and Jorpes 1966, 1968) has also been localized to neuronal perikarya of the ventral midbrain distributed throughout the ventral tegmental area, substantia nigra, and several midline nuclei (Lorén et al. 1979; Innis et al. 1979; Vanderhaeghen et al. 1980). Both immunocytochemical (Hökfelt et al. 1980) and biochemical studies using the neurotoxin 6-hydroxydopamine and/or lesion paradigms (Hökfelt et al. 1980, 1988; Studler et al. 1981, 1984; Williams et al. 1981; Marley et al. 1982; Gilles et al. 1983) have supported the coexistence of CCK and tyrosine hydroxylase (TH, the catecholamine-synthesizing enzyme) within a subpopulation of the ventral mesencephalic neurons. These findings have generated extensive study of this peptide/monoamine coexistence situation. Evidence along several interdisciplinary lines has since accumulated suggesting a functional interaction between CCK and dopamine (see Skirboll et al. 1981; Markstein and Hökfelt 1984; Wang et al. 1984, 1985; Crawley et al. 1985; Hökfelt et al. 1986; Nair et al. 1986).

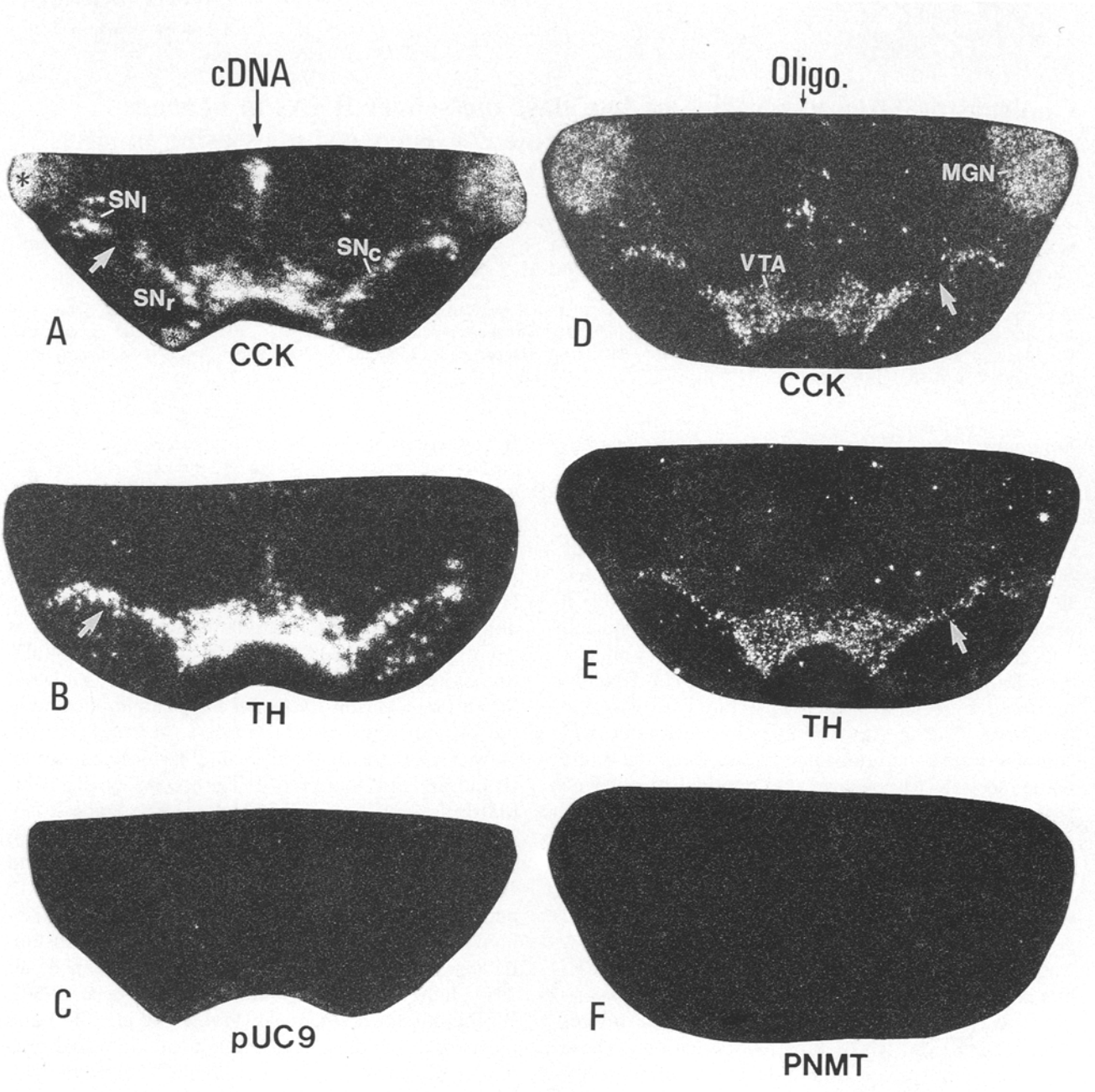

Fig. 1A–F. Film autoradiography. **A–C** Autoradiograms of semi-adjacent coronal sections through the ventral mesencephalon after hybridization with the ^{35}S-labeled cDNA probes for CCK mRNA (**A**), TH mRNA (**B**) or pUC9 (**C**). **D–F** Autoradiograms of adjacent coronal sections through the ventral mesencephalon after hybridization with the ^{35}S-labeled oligonucleotide probes for CCK mRNA (**D**), TH mRNA (**E**) or PNMT mRNA (**F**). Note similar pattern of labeling in ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) and pars lateralis (SNl) in **A** and **B** and in **D** and **E**, respectively. Arrows in these panels indicate corresponding region of the SNc which is labeled for TH mRNA but which lacks labeling for CCK mRNA. Asterisks in **A** denote folds in tissue resulting in non-specific labeling. Note dense labeling over medial geniculate nucleus (MGN) after hybridization with probes for CCK mRNA (A, D), but not with TH mRNA probes (B, E). SNr, substantia nigra pars reticulata. Magnification 10×

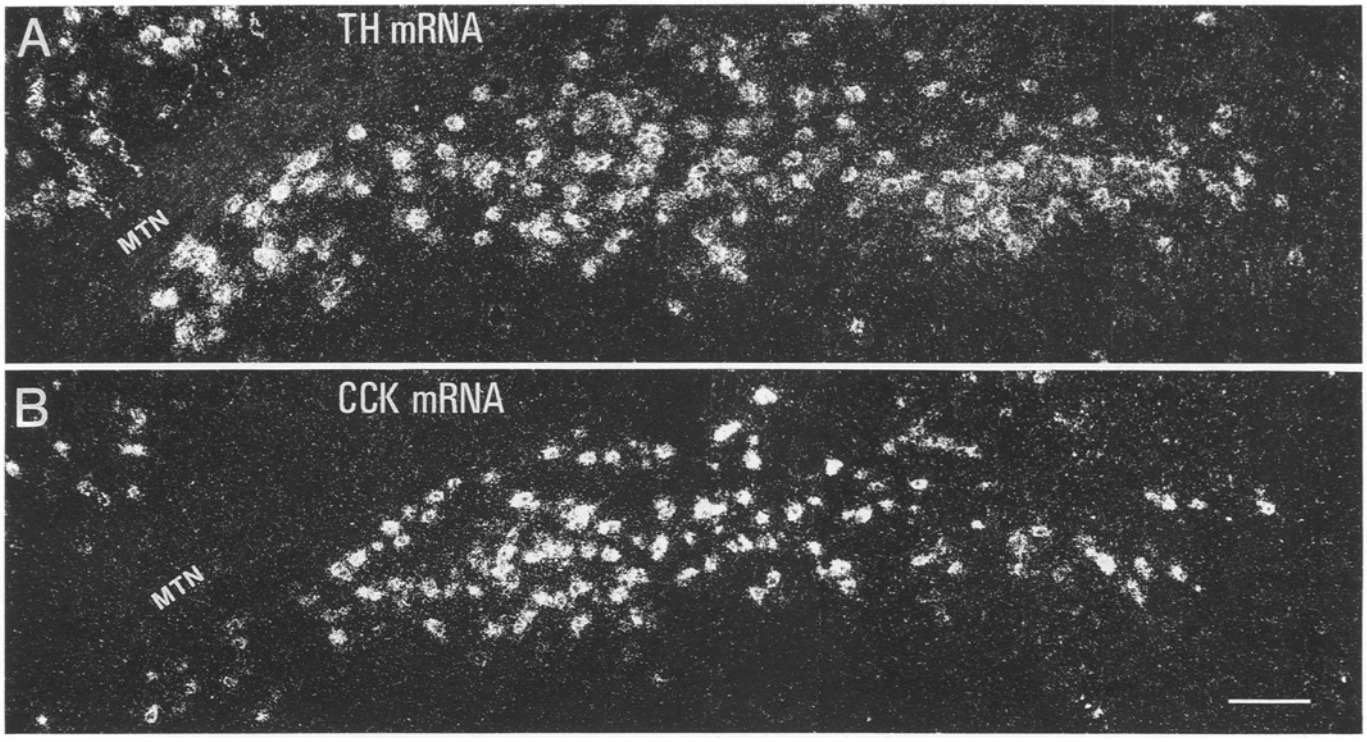

Fig. 2A, B. Darkfield photomicrographs of adjacent sections demonstrating the distribution of neurons (cells) within substantia nigra pars compacta labeled with TH mRNA (**A**) or CCK mRNA (**B**). Oligonucleotide probes. (Unfixed, normal rat.) MTN, medial terminal nucleus of the accessory optic system. Scale bar = 100 μm

Recently, using different antisera, improved fixation techniques, and discrete injections of colchicine (Hökfelt et al. 1986; Seroogy 1986; Seroogy et al. 1987a, 1988; Seroogy and Fallon 1988), a larger population of CCK-containing neurons has been detected in the ventral midbrain as well as a greater incidence of CCK/TH colocalization, particularly in the substantia nigra pars compacta. With the recent cloning and sequence analysis of complementary DNA (cDNA) to CCK messenger RNA (mRNA) of several species (Deschênes et al. 1984; Gubler et al. 1984; Kuwano et al. 1984), and of that to rat TH mRNA (Lamouroux et al. 1982; Grima et al. 1985) as well as the cloning of the rat and human CCK gene (Deschênes et al. 1985; Takahashi et al. 1985), new possibilities for the study of neuronal CCK and TH at the level of gene expression have arisen. In the present study, we have examined the cellular location of neurons in the ventral mesencephalon expressing CCK mRNA and TH mRNA using in situ hybridization methods primarily with cDNA probes. Synthetic oligodeoxyribonucleotide (oligonucleotide) probes were also tested for comparison. In addition, to further analyze the pattern of CCK/TH coexistence at the levels of both mRNA and post-translational product, we have combined the techniques of in situ hybridization and immunocytochemistry on the same tissue section. And finally, since results of recent immunocytochemical studies have questioned the specificity of CCK immunoreactivity in certain systems (Ju et al. 1986, 1987; Hökfelt et al. 1988), the present study also addressed this issue in the ventral mesencephalon at the mRNA level. Parts of these data have been presented in preliminary form (Seroogy et al. 1987b, c).

Material and methods

Animals

Adult male Sprague Dawley rats (150–200 g; ALAB, Stockholm, Sweden) were used in all experiments. Several rats were stereotaxically injected (under 7% (w/v) chloral hydrate anesthesia; i.p.) with colchicine (120 μg in 20 μl 0.9% NaCl) into a lateral ventricle 24–48 h before sacrifice. The animals were deeply anesthetized with sodium pentobarbital and perfused via the ascending aorta with ice-cold formalin prepared from 4% paraformaldehyde in 0.1 M phosphate buffer (PB) (Pease 1962). The brains were removed, immersed in the fixative for 90 min, and placed in 10% sucrose in PB overnight at 4° C. Unperfused animals were also used, in which case the rats were decapitated and the brains quickly dissected out and briefly placed in buffered sucrose. Both unfixed and formalin-fixed brains were cut in a cryostat (Dittes,

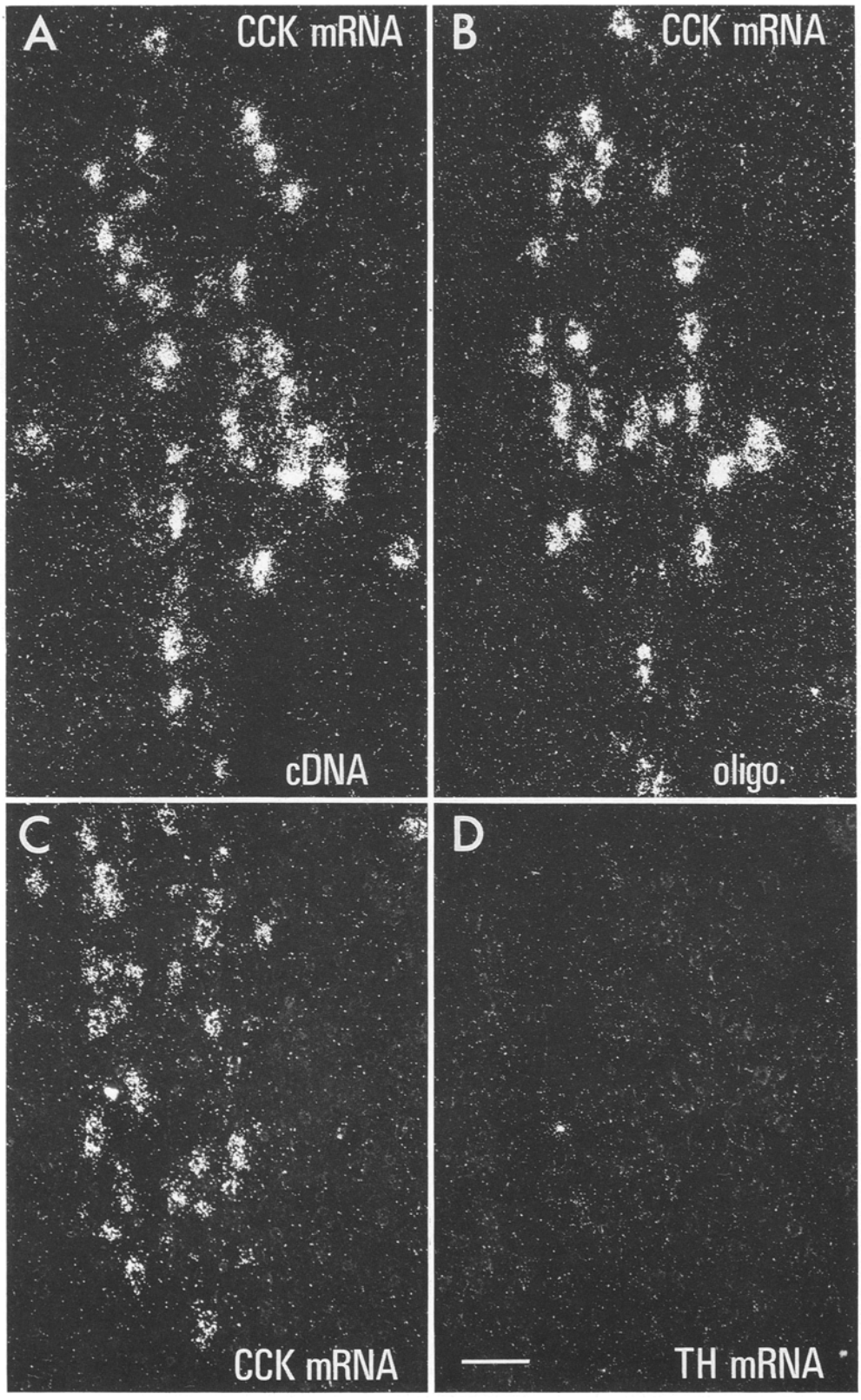

Fig. 3A–D. Darkfield photomicrographs of sections through the ventral periaqueductal grey matter. **A** Cells labeled with the CCK mRNA cDNA probe. (Fixed, colchicine-treated rat.) **B** Cells labeled with the CCK mRNA oligonucleotide probe. (Unfixed, normal rat.) **C** and **D** are adjacent sections showing cells labeled with CCK mRNA (**C**) but not with TH mRNA (**D**). Oligonucleotide probes. (Unfixed, normal rat.) Scale bar = 50 μm

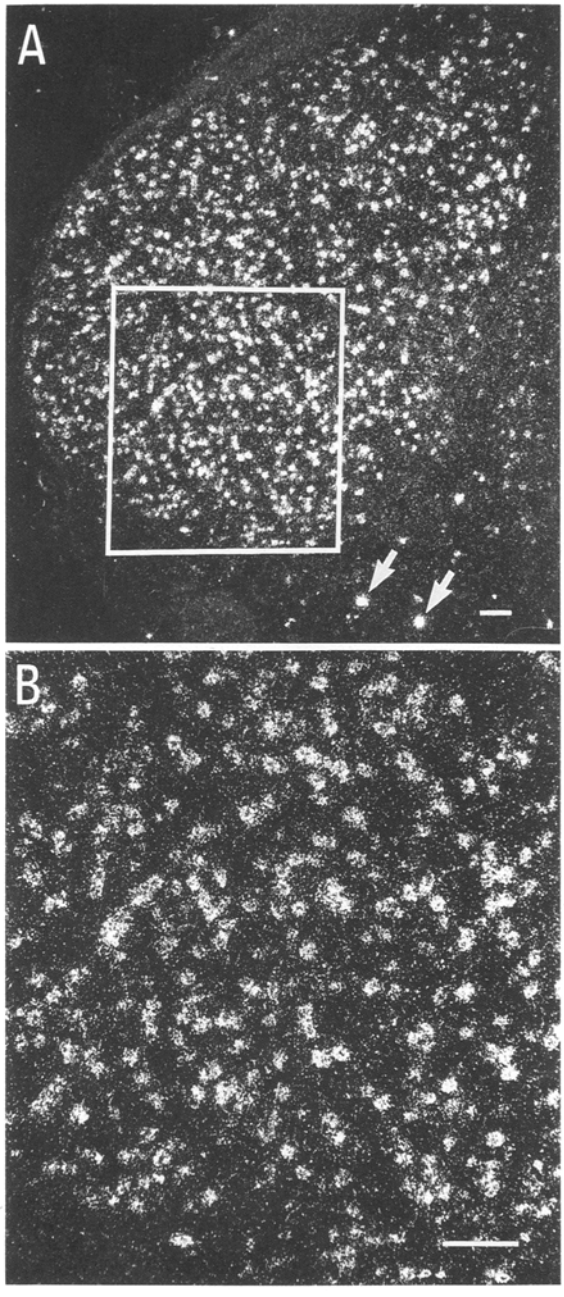

Fig. 4A, B. Darkfield photomicrographs of a section through the medial geniculate nucleus. **A** Low magnification micrograph showing numerous cells labeled with the CCK mRNA oligonucleotide probe. Arrows indicate two large, densely grain-covered cells in the adjacent peripeduncular region. The boxed-in area is shown at higher magnification in **B**. Scale bars = 50 μm

Heidelberg, FRG) in the coronal plane at 14 μm, and sections through the ventral mesencephalon were thaw-mounted onto glass slides treated with 50 μg/ml poly-L-lysine (Sigma, St. Louis, MO, USA) and stored at –70° C overnight or for longer periods. The sections were then processed for in situ hybridization using either cDNA or synthetic oligonucleotide probes (see Young et al. 1986c).

In situ hybridization with cDNA probes and immunocytochemistry

After an initial pretreatment as described in detail previously (Schalling et al. 1986, 1987, 1988), the sections were prehybridized for 4 h at 42° C with a solution containing 50% formamide, 4 × SSC (1 × SSC = 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0), 1 × Denhardt's solution (Maniatis et al. 1982), 1% sarcosyl, 0.02 M $NaPO_4$ (pH 7.0), 10% dextran sulfate, 500 μg/ml yeast tRNA, 37.5 mM dithiothreitol, and 100 μg/ml heat-denaturated salmon sperm DNA. Adjacent sections were then hybridized with the indicated probes. For CCK, a 540 base pair (bp) HindIII/EcoRI fragment derived from a rat preprocholecystokinin cDNA (Deschênes et al. 1984) was used as a probe. The TH probe was a 792 bp PstI fragment derived from a rat TH cDNA (Lamouroux et al. 1982). The DNA fragments were purified by preparative agarose gel electrophoresis and DEAE-cellulose chromatography. Purified DNA fragments were labeled with $\alpha(^{35}S)$-dATP (1300 Ci/mmol; New England Nuclear, Boston, MA, USA) by nick translation to a specific activity of approximately 2×10^8 cpm/μg. The nick translation procedure generated DNA fragments of approximately 100 bases. The plasmid pUC9 was labeled to a similar activity and used as a negative control probe. Hybridization was performed at 42° C for 18 h in a humidified (with 50% formamide in 0.6 M NaCl) chamber using $1–2 \times 10^6$ cpm per slide.

Following hybridization, the sections were rinsed overnight through 5 changes of 0.5 × SSC containing 10 mM sodium thiosulfate, incubated for 1 h at 37° C in the same solution, dipped twice in distilled water, rapidly dried with a stream of air, and placed in a cassette and covered with Hyperfilm β-max X-ray film (Amersham, Amersham, UK) for 1–11 days. Following autoradiographic development of the film (using LX24 developer and AL4 fix; both Kodak, Rochester, NY, USA), the sections were processed for the immunocytochemical localization of CCK or TH using the indirect immunofluorescence technique (see Coons 1958). Briefly, the sections were incubated with either mouse monoclonal anti-CCK antibodies (kindly provided by J. Walsh, C. U. R. E., Los Angeles, CA) (diluted 1 : 400) or rabbit anti-TH antisera (1 : 400) (Markey et al. 1980) overnight at 4° C in a humid atmosphere, rinsed in phosphate buffered saline (PBS), and then incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulins (IgG) or tetramethyl rhodamine isothiocyanate (TRITC)-conjugated goat anti-rabbit IgG (both 1 : 20; Boehringer Mannheim Scandinavia, Stockholm, Sweden), respectively. The sections were rinsed in PBS, coverslipped in a 3 : 1 solution of glycerol : PBS containing 0.1% *p*-phenylenediamine (Johnson and de C. Nogueira Araujo 1981; Platt and Michael 1983) and examined with a Zeiss standard fluorescence microscope equipped with an oil darkfield condensor and proper filter combinations. A KP 500 excitation and a LP 520 barrier filter were used for FITC-fluorescence and a BP 546 excitation and a LP 590 barrier filter for TRITC-fluorescence. After photography with Tri-X black-and-white film (Kodak), the sections were dipped in NTB2 emulsion (Kodak; diluted 1 : 1 with distilled water), exposed for 5–18 days at –20° C, developed with D19 (Kodak) and fixed with G333 (Agfa Gevaert, Leverkusen, FRG), coverslipped in glycerol : PBS, and analyzed in a Leitz Orthoplan light microscope equipped with a darkfield condensor. Finally, the sections were stained with cresyl violet and analysed in the same Leitz microscope under brightfield conditions.

Immunocytochemical controls consisted of preadsorption of the primary antiserum to CCK with 1 μM CCK peptide (generously provided by V. Mutt, Department of Biochemistry, Karolinska Institute, Stockholm, Sweden) or replacement of the TH antiserum with normal rabbit serum. In each case, all immunostaining was absent for the respective antigen.

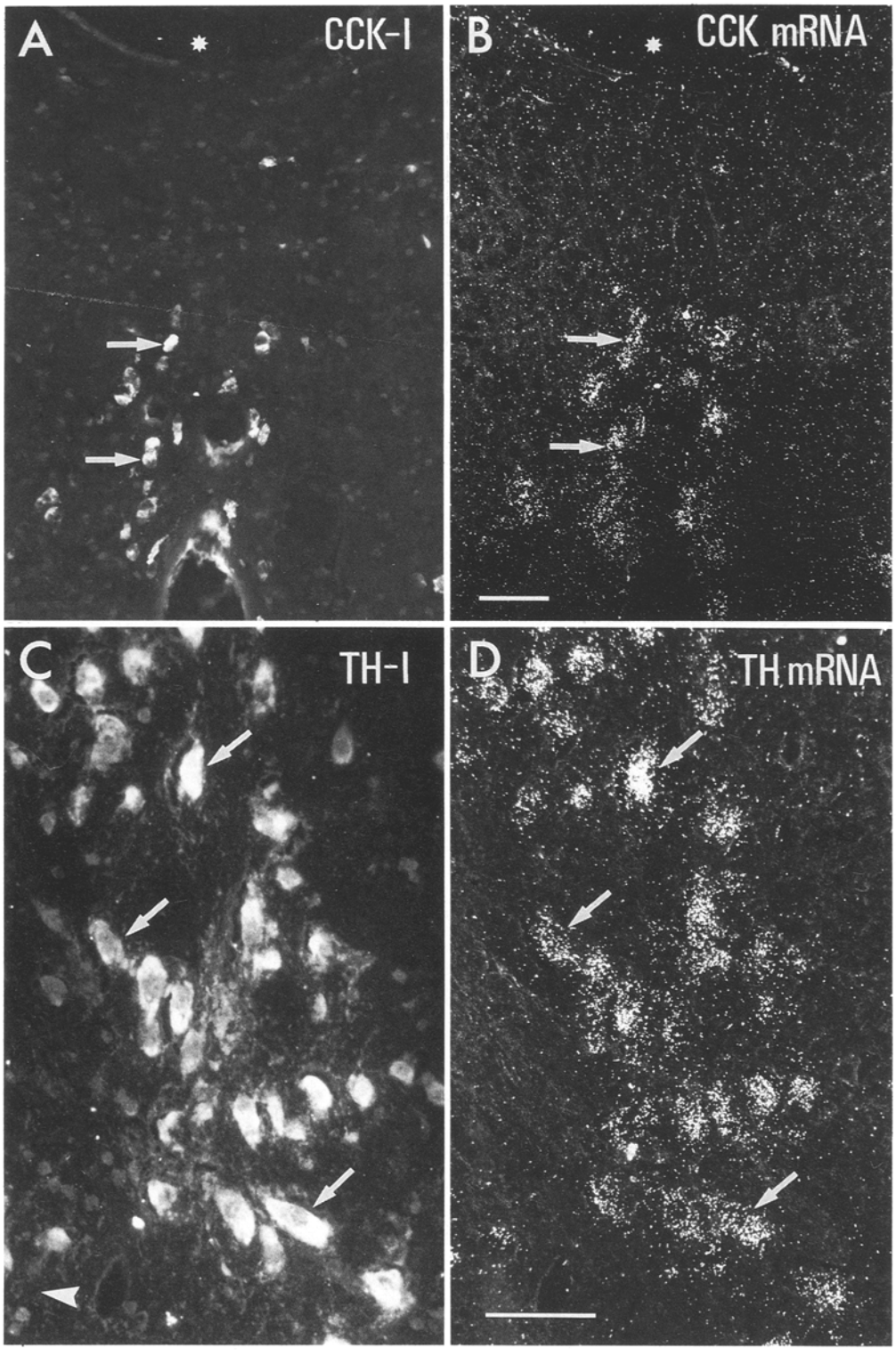

Fig. 5A–D. Combined immunocytochemistry and in situ hybridization. **A**, **B** Are immunofluorescence and darkfield photomicrographs, respectively, of the same section through the ventral periaqueductal grey matter showing cells labeled with both CCK immunoreactivity (**A**) and CCK mRNA (**B**) (cDNA probe). Arrows indicate examples of double-labeled cells. (Fixed, colchicine-treated rat.) **C**, **D** Are immunofluorescence and darkfield photomicrographs, respectively, of the same section of the substantia nigra pars compacta showing cells containing TH immunoreactivity (**C**) and ^{35}S-labeled TH mRNA (**D**) (cDNA probe). Arrows indicate examples of double-labeled cells. (Unfixed, normal rat.) The background immunofluorescence was unusually high in C due to lack of tissue fixation. Arrow head in C indicates donal orientation. Scale bars = 50 μm

In situ hybridization with oligonucleotide probes

Pretreatment of tissue consisted of allowing the slide-mounted sections to equilibrate to room temperature (approximately 30 min), placement in formalin (as above) for 30 min, rinsing twice in phosphate-buffered saline (PBS) for 4 min each, dehydration through a graded series of alcohols and chloroform, and then air-drying. Adjacent sections were then hybridized with the two probes. Hybridization buffer consisted of 50% formamide, 4 × SSC, 1 × Denhardt's solution, 10% dextran sulfate, 250 μg/ml

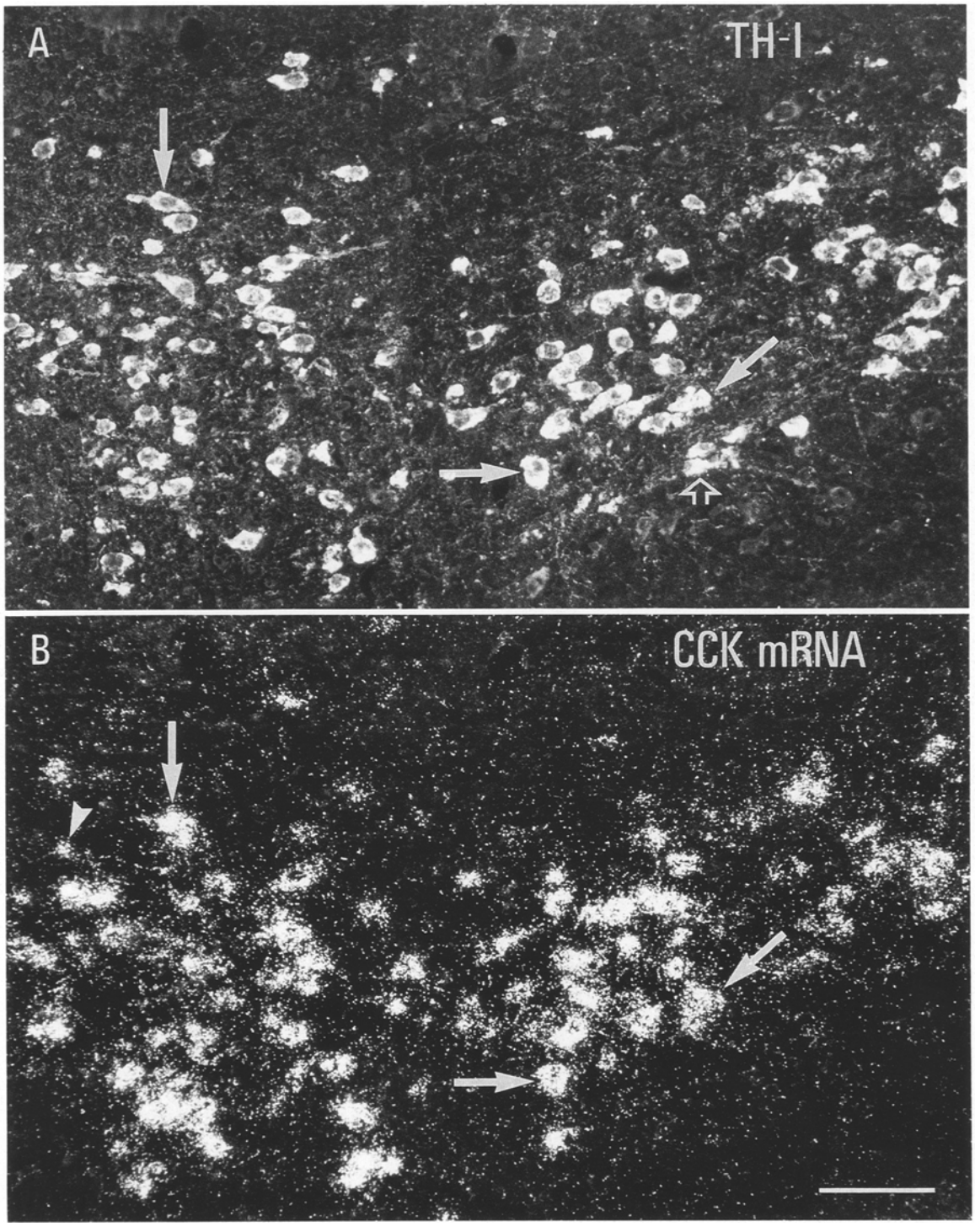

Fig. 6A, B. Combined TH immunocytochemistry and in situ hybridization for CCK mRNA. Immunofluorescence (**A**) and darkfield (**B**) photomicrographs of the same section through the substantia nigra pars compacta showing neurons containing both TH-I (**A**) and ^{35}S-labeled CCK mRNA (**B**) (cDNA probe). (Fixed, colchicine-treated rat.) Arrows indicate examples of double-labeled perikarya. Open arrow in **A** indicates example of TH-I soma which does not contain CCK mRNA. Arrowhead in **B** identifies a CCK mRNA-labeled cell body which lacks TH-I. Scale bar = 50 μm

yeast tRNA, 60 mM dithiothreitol, and 500 μg/ml sheared herring sperm DNA. The 48 base synthetic oligodeoxyribonucleotide probes for CCK (complementary to nucleotides 280–327 of human CCK) (Takahashi et al. 1985) and TH (complementary to nucleotides 1441–1488 of rat TH) (Grima et al. 1985) were made on an AB1 380A DNA synthesizer (Applied Biosystems) and purified on polyacrylamide gels. They were labeled on the 3′ end with α(^{35}S)-dATP using terminal deoxynucleotidyl transferase (IBI, New Haven, CT, USA). A synthetic phenylethanolamine-N-methyltransferase (PNMT) oligonucleotide probe with the same length and specific activity was used as a negative control probe in the ventral mesencephalon. The sections were hybridized with the probes for 18 h at 42° C in a humidified chamber using 1–2 × 10^6 cpm/slide.

Following hybridization, the sections were rinsed in 1 × SSC (4 times for 15 min each) at 55° C and for 1 h at room temperature, dipped twice in distilled water, dried with a stream of air, placed in a cassette, and then processed for X-ray film and emulsion autoradiography as described above.

RNA blot hybridization

Total or poly(A)$^+$ RNA was prepared from the pooled ventral mesencephalons of 10 rats as described elsewhere (Whittemore et al. 1986). In brief, the tissue was homogenized in 4 M guanidine isothiocyanate and centrifuged for 14 h at 35000 rpm on a cushion of 5.7 M CsCl. The pellet was resuspended and phenol/chloroform extracted twice before ethanol precipitation. Fifteen μg of total or poly(A)$^+$ RNA was run on 1% agarose gels containing 0.7% formaldehyde and blotted onto nitrocellulose filters overnight. The filter-bound RNAs were initially hybridized to a rat TH cDNA probe labeled with α(^{32}P)-dCTP by nick translation. After hybridization, the filters were washed through several changes of 0.1 × SSC/1% sodium dodecyl sulfate at 54° C and then exposed to XAR-5 X-ray film (Kodak) with intensifying screens overnight. The filters were subsequently boiled for 5 min in 1% glycerol and then probed with a nick-translated rat CCK cDNA probe as above. Finally, the filters were boiled again and hybridized with a nick-translated probe encoding rat α-actin for control purposes.

Results

Film autoradiography

After hybridization of sections with the ^{35}S-labeled cDNA or oligonucleotide probes, autoradiograms were obtained demonstrating the presence of CCK mRNA and TH mRNA throughout several regions of the ventral mesencephalon (Fig. 1). Similar results were obtained with either the cDNA probes or the oligonucleotide probes. The same basic pattern was observed for both CCK mRNA and TH mRNA in significant portions of the ventral tegmental area and substantia nigra pars compacta and lateralis in adjacent or semi-adjacent sections. However, at intermediate and especially at caudal levels of the ventral midbrain, it was apparent that whereas labeling indicating TH mRNA extended the entire mediolateral extent of the substantia nigra pars compacta, labeling for CCK mRNA was not present in the most lateral part of this structure, just medial to the substantia nigra pars lateralis. This is most clearly illustrated in Fig. 1A, D. Generally, the intensity of labeling was lower with the CCK mRNA probes as compared to that of the TH mRNA probes. Therefore, the slides processed with the CCK mRNA probes were often exposed to the autoradiography film for a longer time than slides treated with the TH mRNA probes (see Fig. 1). The longer CCK mRNA probe exposure times were also necessary with the liquid emulsion autoradiographic processing of the slides (see below).

In other regions, a marked differential distribution of TH mRNA and CCK mRNA was observed. These included the substantia nigra pars reticulata which exhibited labeling only for TH mRNA (Fig. 1B, E) and an intensely labeled group along the midline expressing almost exclusively CCK mRNA (Fig. 1A, D). Labeling for CCK mRNA but never for TH mRNA was observed in the medial geniculate nucleus (Fig. 1A, D). No specific labeling above background was seen in autoradiograms of adjacent ventral midbrain sections hybridized with the pUC9 control probe (Fig. 1C) or with the PNMT probe (Fig. 1F).

Emulsion autoradiography

Following liquid emulsion autoradiography, neurons throughout the ventral tegmental area (A10), substantia nigra pars compacta (A9) and pars lateralis, interfascicular nucleus, rostral and caudal linear nuclei, and central grey matter adjacent to the aqueduct were found to contain CCK mRNA and TH mRNA as indicated by clusters of grains over cell bodies in these structures using the respective ^{35}S-labeled cDNA or oligonucleotide probes. Figure 2 shows adjacent sections through the substantia nigra pars compacta, in which neurons expressing TH mRNA or CCK mRNA exhibited a very similar pattern of distribution. This was the case from rostral to intermediate levels of the ventral mesencephalon. At more caudal levels, CCK mRNA-labeled cells were absent from the lateral substantia nigra pars compacta. It was apparent that at all levels higher numbers of neurons expressing TH mRNA relative to CCK mRNA were present in the substantia nigra pars compacta. Large neurons expressing only CCK mRNA were observed in the ventral periaqueductal grey matter (including the Edinger-Westphal nucleus) (Fig. 3). Those expressing only TH mRNA were located in the substantia nigra pars reticulata with the exception of the presence of extremely rare CCK mRNA-labeled cells. In sections processed with the probes for CCK mRNA, clusters of grains were

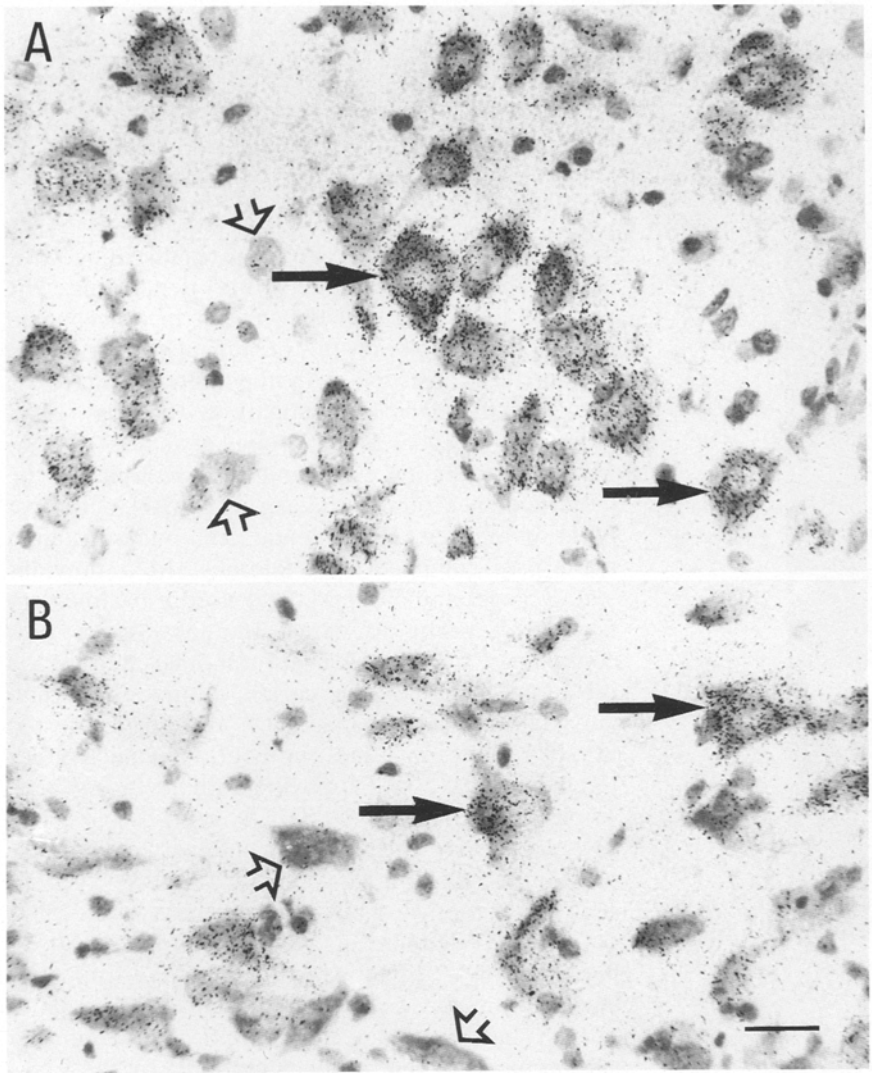

Fig. 7A, B. Brightfield photomicrographs of neurons in the ventral periaqueductal grey matter (**A**) and substantia nigra pars compacta (**B**) labeled for CCK mRNA (oligonucleotide probe). Arrows indicate examples of somata covered with clusters of grains whereas the open arrows indicate examples of unlabeled cell bodies. (Unfixed, normal rat.) Scale bar = 25 μm

present over numerous cell bodies in the medial geniculate nucleus (Fig. 4). No clusters of grains indicating specific labeling of cell bodies in any of these or any other regions were observed in sections processed with the pUC9 probe or PNMT probe.

Combined in situ hybridization and immunocytochemistry

Immunoreactivity for either the peptide or the enzyme was localized within their respective perikarya; i.e. CCK-like immunoreactivity (CCK-I) was detected within cells labeled for CCK mRNA (Fig. 5A, B) and TH-I was observed in neurons containing TH mRNA (Fig. 5C, D). Additionally, the presence of TH-I (Fig. 6A) was demonstrated within cell bodies labeled for CCK mRNA (Fig. 6B) and CCK-I was found in TH mRNA-labeled perikarya. Note that in Fig. 6B, the grain-labeled cells extended into the middle to lateral regions of the substantia nigra pars compacta and that there was substantial double-labeling of CCK mRNA-labeled (Fig. 6B) and TH immunostained (Fig. 6A) somata. However, single-labeled TH-I neurons were consistently observed not covered with grain clusters (see Fig. 6A), while an infrequent CCK mRNA-labeled cell appeared to lack immunoreactivity for the enzyme (see Fig. 6B). The large cell bodies of the ventral periaqueductal grey matter, which were CCK mRNA-positive (Figs. 3, 5B), were always TH-I negative.

It should be noted that whereas immunoreactivity for TH was detected in ventral mesencephalic neurons of both fixed and unfixed tissue and in either non-treated or colchicine-treated animals, CCK immunostaining could only be observed in formalin-fixed, colchicine-treated rats. Moreover, this CCK-I was weak and usually limited to neurons in the ventral tegmental area, midline nuclei, and most medial substantia nigra pars compacta. Thus, CCK-I/

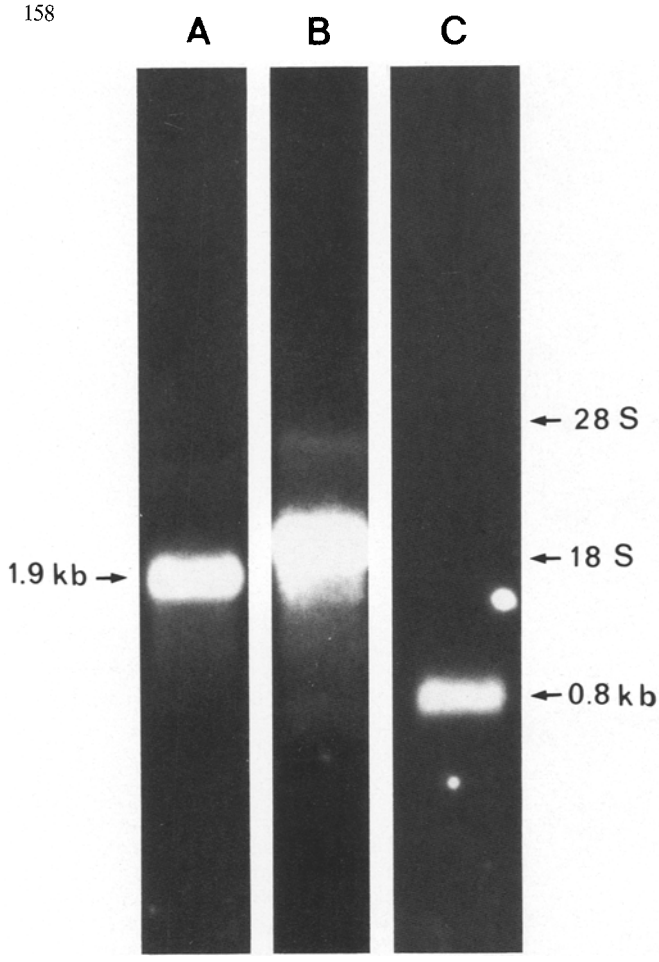

Fig. 8A–C. Film autoradiograms from an RNA blot hybridization experiment. **A–C** Show the same lane. Poly $(A)^+$ RNA extracted from the ventral mesencephalon was run on a 1% agarose gel, blotted onto a nitrocellulose filter and hybridized initially with a nick-translated rat TH cDNA probe (**A**). The filter was then boiled for 10 min and rehybridized with a rat CCK cDNA probe (**C**). For comparison the filter was again rehybridized with actin (**B**)

TH mRNA double-labeling could only be observed in these regions. In addition, immunoreactivity for the CCK peptide was never observed in neurons of the medial geniculate nucleus.

Analysis of cresyl violet-stained sections under brightfield conditions revealed that high grain densities were, in all regions examined, always and exclusively observed over cell body profiles exhibiting neuronal characteristics (Fig. 7).

RNA blot analysis

RNA blot analyses were performed in order to confirm the presence of mature mRNA for TH and CCK, respectively, in the ventral mesencephalon, as well as to verify the specificity of the cDNA probes. Hybridization with the rat TH cDNA probe showed a single TH mRNA with the expected size of 1.9 kb (Lamouroux et al. 1982) (Fig. 8A). The CCK probe also hybridized to a single mRNA species with the expected size of 0.8 kb (Deschênes et al. 1984) (Fig. 8C).

Discussion

The present in situ hybridization results using CCK cDNA and oligonucleotide probes demonstrate the presence of neurons in the ventral mesencephalon that express mRNA for this peptide. The distribution obtained is consistent with the more recent CCK immunocytochemical investigations (Seroogy 1986; Hökfelt et al. 1986, 1988; Seroogy et al. 1987a, 1988; Seroogy and Fallon 1988) and is very similar to the pattern of neurons expressing TH mRNA in most parts of the ventral tegmental area and substantia nigra pars compacta and lateralis. In a previous study, Siegel and Young (1985) used a complementary RNA probe to detect the location of cells containing CCK mRNA in several other areas of the rat brain including the cortex, hippocampus and amygdala. Similar findings have also been reported in abstract form using synthetic oligonucleotides (Réthelyi et al. 1986). Several other studies have employed ^{32}P-labeled CCK cDNA probes in RNA blot analyses of the developmental or regional expression of CCK mRNA in rat (Hasegawa et al. 1986; Duchemin et al. 1987; Bönnemann et al. 1987) or mouse (Friedman et al. 1985) brain. Although most of these investigations examined primarily whole brain or large regional dissections, CCK mRNA has been detected specifically within the substantia nigra/ventral tegmental area by the RNA blot hybridization technique (Bönnemann et al. 1987), as was also demonstrated in the present study.

The present localization of TH mRNA-containing perikarya throughout this region using the TH cDNA probe and oligonucleotide probe is in good agreement with previous studies using complementary RNA probes for TH (Schalling et al. 1986; Chesselet et al. 1987) and with other recent studies using oligonucleotide TH probes (Young et al. 1986a; Han et al. 1987) or a TH cDNA probe (Berod et al. 1987). The distribution of these neurons expressing TH mRNA corresponds closely with the pattern of TH immunoreactivity (indicative of dopamine neurons) obtained in this study and that which has been well-documented for mesencephalic dopamine neurons in other studies (see Dahlström and Fuxe 1964; Björklund and Lindvall 1984; Hökfelt et al. 1984a).

By combining the methods of in situ hybridization and immunocytochemistry on the same tissue section (see Gendelman et al. 1985; Wolfson et al. 1985; Shivers et al. 1986; Schalling et al. 1986; Siegel

and Young 1986; Young et al. 1986b), we were able to determine that not only did CCK mRNA- and TH mRNA-positive neurons display immunoreactivity for their respective post-translational product, but also that cell bodies labeled for CCK mRNA contained the TH enzyme and that many expressing TH mRNA contained the CCK peptide. These results are in agreement with previous double-labeling immunocytochemical studies (Hökfelt et al. 1980; Seroogy 1986; Seroogy et al. 1988) and suggest significant CCK/dopamine coexistence in the ventral mesencephalon, including the substantia nigra pars compacta. Thus, this combined hybridization histochemical/immunocytochemical technique can provide another approach in determining coexistence of different putative neurotransmitters/neuromodulators. This could be particularly advantageous in cases where one of the substances is difficult to visualize immunocytochemically without the use of colchicine. As shown in this study, neurons containing CCK mRNA could be demonstrated in normal as well as colchicine-treated rats, whereas the peptide immunoreactivity was only detected in the colchicine-injected animals, and not to the extent that was seen for the CCK mRNA-labeled cells. It should be noted that the CCK immunostaining obtained was always weak, even in these colchicine-treated rats. This was presumably due to the harsh treatment incurred during the hybridization procedure, which preceded the incubation with antisera. In contrast, the TH immunostaining was always generally robust possibly due to the "potency" of this antiserum and/or a better resistence of the enzyme to the hybridization procedure. Thus, in certain circumstances, the in situ hybridization technique obviates the need for colchicine and can be a more reliable, sensitive indicator of peptide (or other substance)-containing neurons.

Evidence for the specificity of the probes used in these experiments is provided by the observation that no labeling above background was obtained with a control plasmid vector cDNA probe (not containing CCK or TH sequences) or with an oligonucleotide probe for the epinephrine-synthesizing enzyme PNMT, which is not present in cell bodies in ventral mesencephalic regions (Hökfelt et al. 1984b). Both the cDNA probe and the oligonucleotide probe for CCK mRNA gave identical results as did the two different probes for TH mRNA. In addition, as mentioned above, the pattern of perikaryal grain-labeling obtained with both the cDNA probes and the oligonucleotide probes for CCK and TH in the ventral mesencephalon is in very close agreement with the distribution of CCK and TH immunostaining described in immunocytochemical studies. Finally, our RNA blot analysis of polyadenylated or total RNA from the rat ventral mesencephalon with the cDNA probes revealed only single hybridizing mRNAs for CCK and TH.

Recently, the nature of immunoreactive CCK has been questioned in several regions of the rat nervous system. It appears that antisera directed against the COOH-terminal region of the CCK peptide in the rat may be cross-reacting with and thus actually detecting calcitonin gene-related peptide (CGRP) in systems such as the dorsal root ganglia and their projections to the dorsal horn of the spinal cord (Ju et al. 1986, 1987; Hökfelt et al. 1988). In contrast, the present CCK mRNA in situ and RNA blot hybridization results suggest strongly that neuronal perikarya in the ventral mesencephalon represent "true" CCK neurons.

As mentioned previously, the results obtained with the CCK mRNA probes in the MGN are not in accordance with CCK immunocytochemical data. Despite the use of colchicine and several different sequence-specific antisera, CCK cell bodies have never been demonstrated within this thalamic nucleus (see Innis et al. 1979; Vanderhaeghen et al. 1980; Cho et al. 1983; Hunt et al. 1987; Hökfelt et al. 1988). There may be several possibilities for this apparent discrepancy between the presence of numerous CCK mRNA-containing perikarya and the lack of observable CCK immunoreactive somata. Firstly, the peptide may be present within the cell bodies but in amounts below the limits of sensitivity of present immunocytochemical techniques. Secondly, the peptide may be synthesized but then rapidly exported from the soma to the axon, although this should be prevented by colchicine treatment. Thirdly, the CCK mRNA present within the MGN cells might remain untranslated. Fourthly, the CCK message may be translated but the subsequent product may be processed differently than in other neurons, in such a manner as to render it unrecognizable using presently available antisera. Differential post-translational modification of CCK has been recently reported in endocrine cells of the adenohypophysis (Rehfeld 1987). Finally, the CCK mRNA probes could be cross-hybridizing with mRNAs unrelated to CCK. With regard to the latter, it is of course realized that additional controls for both CCK mRNA probes used in these experiments are necessary before an unequivocal statement can be made concerning the specificity of the MGN CCK mRNA-labeled neurons. It should be noted, however, that other recent studies of mRNAs for CCK or other peptides have also found similar discrepancies between the distribution of labeled perikarya obtained with in situ hybridization versus immuno-

cytochemical techniques (Siegel and Young 1985; Harlan et al. 1987; Segerson et al. 1987).

The present results are pertinent to the numerous investigations of the functional interactions of CCK and dopamine in the mesotelencephalic system. Many inconsistencies have been reported among different physiological, pharmacological and behavioral studies (see extensive reviews of literature in Nair et al. 1986 and Seroogy 1986), especially concerning the facilitatory versus inhibitory effects of CCK upon several dopamine-mediated functions (Schneider et al. 1983; Widerlöv et al. 1983; Hamilton et al. 1984; Markstein and Hökfelt 1984; White and Wang 1984; Crawley et al. 1984, 1985; Wang et al. 1985; Wang and Hu 1986; Blumstein et al. 1987). The extent of CCK/dopamine perikaryal colocalization in the ventral mesencephalon has entered into the interpretation of some of these data. The present combined in situ hybridization and immunocytochemical study provides additional evidence for a higher degree of CCK/dopamine coexistence within neurons of the ventral midbrain (most notably in the substantia nigra pars compacta) than previously thought. The present results, thus, provide strong evidence that mesencephalic dopamine neurons in fact produce CCK and that CCK/dopamine coexistence may have implications, for example, in the neuropathology of schizophrenia (Roberts et al. 1983; Verbanck et al. 1984; Wang et al. 1984; Nair et al. 1986) and perhaps Parkinson's disease (Studler et al. 1982; Studler and Javoy-Agid 1985).

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References

Berod A, Faucon Biguet N, Dumas S, Bloch B, Mallet J (1987) Modulation of tyrosine hydroxylase gene expression in the central nervous system visualized by in situ hybridization. Proc Natl Acad Sci USA 84: 1699–1703

Björklund A, Lindvall O (1984) Dopamine-containing systems in the CNS. In: Björklund A, Hökfelt T (eds) Handbook of chemical neuroanatomy, Vol 2. Classical transmitters in the CNS, Part I. Elsevier, Amsterdam, pp 55–122

Blumstein LK, Crawley JN, Davis LG, Baldino F Jr (1987) Neuropeptide modulation of apomorphine-induced stereotyped behavior. Brain Res 404: 293–300

Bruyn GW (1968) Huntington's chorea: historical, clinical and laboratory synopsis. In: Vinkin PJ, Bruyn GW (eds) Handbook of clinical neurology, Vol 6. Diseases of the basal ganglia. North Holland, Amsterdam, pp 298–378

Bönnemann C, Giraud P, Eiden LE, Meyer DK (1987) Measurement of mRNA specific for preprocholecystokinin in rat caudatoputamen and areas projecting to it. Neurochem Int 10: 521–524

Chesselet M-F, Weiss L, Wuenschell C, Tobin AJ, Affolter HU (1987) Comparative distribution of mRNAs for glutamic acid decarboxylase, tyrosine hydroxylase, and tachykinins in the basal ganglia: an in situ hybridization study in the rodent brain. J Comp Neurol 262: 125–140

Cho HJ, Shiotani Y, Shiosaka S, Inagaki S, Kubota Y, Kiyama H, Umegaki K, Tateishi K, Hashimura E, Hamaoka T, Tohyama M (1983) Ontogeny of cholecystokinin-8-containing neuron system of the rat: an immunohistochemical analysis. I. Forebrain and upper brainstem. J Comp Neurol 218: 25–41

Coons AH (1958) Fluorescent antibody methods. In: Danielli JF (ed) General cytochemical methods. Academic Press, New York, pp 399–422

Crawley JN, Hommer DW, Skirboll LR (1984) Behavioral and neurophysiological evidence for a facilitatory interaction between co-existing transmitters: cholecystokinin and dopamine. Neurochem Int 6: 755–760

Crawley JN, Stivers JA, Blumstein LK, Paul SM (1985) Cholecystokinin potentiates dopamine-mediated behaviors: evidence for modulation specific to a site of coexistence. J Neurosci 5: 1972–1983

Dahlström A, Fuxe K (1964) Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. Acta Physiol Scand [Suppl] 62: 1–55

Deschênes RJ, Lorenz LJ, Haun RS, Roos BA, Collier KJ, Dixon JE (1984) Cloning and sequence analysis of a cDNA encoding rat preprocholecystokinin. Proc Natl Acad Sci USA 81: 726–730

Deschênes RJ, Haun RS, Funckes CL, Dixon JE (1985) A gene encoding rat cholecystokinin: isolation, nucleotide sequence, and promotor activity. J Biol Chem 260: 1280–1286

Duchemin AM, Quach TT, Iadarola MJ, Deschênes RJ, Schwartz JP, Wyatt RJ (1987) Expression of the cholecystokinin gene in rat brain during development. Dev Neurosci 9: 61–67

Fink JS, Smith GP (1980) Mesolimbicocortical dopamine terminal fields are necessary for normal locomotor and investigatory exploration in rats. Brain Res 199: 359–384

Friedman J, Schneider BS, Powell D (1985) Differential expression of the mouse cholecystokinin gene during brain and gut development. Proc Natl Acad Sci USA 82: 5593–5597

Gendelman HE, Moench TR, Narayan O, Griffin DE, Clements JE (1985) A double labeling technique for performing immunocytochemistry and in situ hybridization in virus infected cell cultures and tissues. J Virol Methods 11: 93–103

Gilles C, Lotstra F, Vanderhaeghen J-J (1983) CCK nerve terminals in rat striatum and limbic areas originate partly in the brain stem and partly in telencephalic areas. Life Sci 32: 1683–1690

Grima B, Lamouroux A, Blanet F, Faucon Biguet N, Mallet J (1985) Complete coding sequence of rat tyrosine hydroxylase mRNA. Proc Natl Acad Sci USA 82: 617–621

Gubler U, Chua AO, Hoffman BJ, Collier KJ, Eng J (1984) Cloned cDNA to cholecystokinin mRNA predicts an identical preprocholecystokinin in pig brain and gut. Proc Natl Acad Sci USA 81: 4307–4310

Hamilton M, Sheehan MJ, De Belleroche J, Herberg LJ (1986) The cholecystokinin analogue, caerulein, does not modulate dopamine release or dopamine-induced locomotor activity in the nucleus accumbens of rat. Neurosci Lett 44: 77–82

Han VKM, Snouweart J, Towle AC, Lund PK, Lauder JM (1987) Cellular localization of tyrosine hydroxylase mRNA and its regulation in the rat adrenal medulla and brain by in situ

hybridization with an oligodeoxyribonucleotide probe. J Neurosci Res 17: 11–18

Harlan RE, Shivers BD, Romano GJ, Howells RD, Pfaff DW (1987) Localization of preproenkephalin mRNA in the rat brain and spinal cord by in situ hybridization. J Comp Neurol 258: 159–184

Hasegawa M, Usui H, Araki K, Kuwano R, Takahashi Y (1986) Developmental and regional changes of cholecystokinin mRNA in rat brain. FEBS Lett 194: 224–226

Hökfelt T, Skirboll L, Rehfeld JF, Goldstein M, Markey K, Dann O (1980) A subpopulation of mesencephalic dopamine neurons projecting to limbic areas contains a cholecystokinin-like peptide: evidence from immunohistochemistry combined with retrograde tracing. Neuroscience 5: 2093–2124

Hökfelt T, Mårtensson R, Björklund A, Kleinau S, Goldstein M (1984a) Distributional maps of tyrosine hydroxylase-immunoreactive neurons in the rat brain. In: Björklund A, Hökfelt T (eds) Handbook of chemical neuroanatomy, Vol 2. Classical transmitters in the CNS, Part I. Elsevier, Amsterdam, pp 277–379

Hökfelt T, Johansson O, Goldstein M (1984b) Central catecholamine neurons as revealed by immunohistochemistry with special reference to adrenaline neurons. In: Björklund A, Hökfelt T (eds) Handbook of chemical neuroanatomy, Vol 2. Classical transmitters in the CNS, Part I. Elsevier, Amsterdam, pp 157–276

Hökfelt T, Holets VR, Staines W, Meister B, Melander T, Schalling M, Schultzberg M, Freedman J, Björklund H, Olson L, Lindh B, Elfrin L-G, Lundberg JM, Lindgren JÅ, Samuelsson B, Pernow B, Terenius L, Post C, Everitt B, Goldstein M (1986) Coexistence of neuronal messengers – an overview. Prog Brain Res 68: 33–70

Hökfelt T, Herrera-Marschitz M, Seroogy K, Ju G, Holets V, Schalling M, Ungerstedt U, Post C, Rehfeld JF, Frey P, Fischer J, Dockray G, Hamaoka T, Walsh JH, Goldstein M (1988) Immunohistochemical studies on cholecystokinin (CCK)-immunoreactive neurons in the rat using sequence-specific antisera and with special reference to the caudate nucleus and primary sensory neurons. J Chem Neuroanat 1: 11–52

Hornykiewicz O (1980) Biochemical abnormalities in some extrastriatal neuronal systems in Parkinson's disease. In: Rinne UK, Klinger M, Stan G (eds) Parkinson's disease, current progress, problems and management. Elsevier, Amsterdam, pp 109–119

Hunt CA, Seroogy KB, Gall CM, Jones EG (1987) Cholecystokinin innervation of rat thalamus, including fibers to ventroposterolateral nucleus from dorsal column nuclei. Mol Brain Res 426: 257–269

Innis RB, Correa FM, Uhl GR, Schneider B, Snyder SH (1979) Cholecystokinin octapeptide-like immunoreactivity: histochemical localization in rat brain. Proc Natl Acad Sci USA 76: 521–525

Javoy-Agid F, Ruberg M, Tequet H, Bokobza B, Agid Y, Gaspar P, Breger B, N'Guyen-Legros J, Alvarez C, Gray F, Escourolle R, Scatton B, Rouquier L (1984) Biochemical neuropathology of Parkinson's disease. Adv Neurol 40: 189–198

Johnson DG, de C Nogueira Araujo GM (1981) A simple method of reducing the fading of immunofluorescence during microscopy. J Immunol Methods 43: 349–350

Ju G, Hökfelt T, Fischer JA, Frey P, Rehfeld JF, Dockray GJ (1986) Does cholecystokinin-like immunoreactivity in rat primary sensory neurons represent calcitonin gene-related peptide? Neurosci Lett 68: 305–310

Ju G, Hökfelt T, Brodin E, Fahrenkrug J, Fischer JA, Frey P, Elde RP, Brown JC (1987) Primary sensory neurons of the rat showing calcitonin gene-related peptide (CGRP) immunoreactivity and their relation to substance P-, somatostatin-, galanin-, vasoactive intestinal polypeptide- and cholecystokinin-immunoreactive ganglion cells. Cell Tissue Res 247: 417–431

Kuwano R, Araki K, Usui H, Fukui T, Ohtsuka E, Ikehara M, Takahashi Y (1984) Molecular cloning and nucleotide sequence of cDNA coding for rat brain cholecystokinin precursor. J Biochem 96: 923–926

Lamouroux A, Faucon Biguet N, Samolyk D, Privat A, Salomon JC, Pujol JF, Mallet J (1982) Identification of cDNA clones coding for rat tyrosine hydroxylase antigen. Proc Natl Acad Sci USA 79: 3881–3885

Lindvall O, Björklund A (1983) Dopamine- and norepinephrine-containing neuron systems: their anatomy in the rat brain. In: Emson PC (ed) Chemical neuroanatomy. Raven Press, New York, pp 229–255

Lorén I, Alumets J, Håkanson R, Sundler F (1979) Distribution of gastrin and CCK-like peptides in rat brain: an immunocytochemical study. Histochemistry 59: 249–257

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor

Markey KA, Kondo S, Shenkman L, Goldstein M (1980) Purification and characterization of tyrosine hydroxylase from a clonal pheochromocytoma cell line. Mol Pharmacol 17: 79–85

Markstein R, Hökfelt T (1984) Effect of cholecystokinin-octapeptide on dopamine release from slices of cat caudate nucleus. J Neurosci 4: 570–575

Marley PD, Emson PC, Rehfeld JH (1982) Effect of 6-hydroxydopamine lesions of the medial forebrain bundle on the distribution of cholecystokinin in rat forebrain. Brain Res 252: 382–385

Mason ST (1984) Catecholamines and behavior. Cambridge University Press, Cambridge

Matthysse SW, Kety SS (1975) Catecholamines and schizophrenia. Pergamon Press, Oxford

Meltzer HY, Stahl SM (1976) The dopamine hypothesis of schizophrenia: a review. Schizophr Bull 2: 19–76

Mutt V, Jorpes JE (1966) Isolation of aspartyl-phenylalanine amide from cholecystokinin-pancreozymin. Biochem Biophys Res Commun 26: 392–397

Mutt V, Jorpes JE (1968) Structure of porcine cholecystokinin-pancreozymin. I. Cleavage with thrombin and with trypsin. Eur J Biochem 6: 156–162

Nair NPV, Lal S, Bloom DM (1986) Cholecystokinin and schizophrenia. Prog Brain Res 65: 237–258

Pease PC (1962) Buffered formaldehyde as a killing agent and primary fixative for electron microscopy. Anat Rec 142: 342

Platt JL, Michael AF (1983) Retardation of fading and enhancement of intensity of immunofluorescence by p-phenylenediamine. J Histochem Cytochem 31: 840–842

Randrup A, Munkvad I (1972) Evidence indicating an association between schizophrenia and dopaminergic hyperactivity in the brain. Orthomol Psychiatr 1: 2–7

Rehfeld JF (1987) Preprocholecystokinin processing in the normal human anterior pituitary. Proc Natl Acad Sci USA 84: 3019–3023

Réthelyi M, McGehee D, Lund PK (1986) Neuronal localization of cholecystokinin mRNAs in rat and guinea pig. Soc Neurosci Abstr 12: 1041

Roberts GW, Ferrier IN, Lee Y, Crow TJ, Johnstone EC, Owens DGC, Bacarese-Hamilton AJ, McGregor G, O'Shaughnessy D, Polak JM, Bloom SR (1983) Peptides, the limbic lobe and schizophrenia. Brain Res 288: 199–211

Schalling M, Hökfelt T, Wallace B, Goldstein M, Filer D, Yamin C, Schlessinger DH (1986) Tyrosine 3-hydroxylase in rat brain

and adrenal medulla: hybridization histochemistry and immunohistochemistry combined with retrograde tracing. Proc Natl Acad Sci USA 83: 6208–6212

Schalling M, Dagerlind Å, Brené S, Petterson R, Carrol JM, Joh TH, Hökfelt T (1987) Localization of mRNA for phenylethanolamine-N-methyltransferase (PNMT) using in situ hybridization. Acta Physiol Scand 131: 631–632

Schalling M, Seroogy K, Hökfelt T, Chai SY, Hallman H, Persson H, Larhammar D, Ericsson A, Terenius L, Graffi J, Massoulié J, Goldstein M (1988) Neuropeptide Y in the rat adrenal gland – immunohistochemical and in situ hybridization studies. Neuroscience 24: 337–349

Schneider LH, Albert JE, Iversen SD (1983) CCK-8 modulation of mesolimbic dopamine: antagonism of amphetamine-stimulated behaviors. Peptides 4: 749–753

Segerson TP, Hoefler H, Childers H, Wolfe HJ, Wu P, Jackson IMD, Lechan RM (1987) Localization of thyrotropin-releasing hormone prohormone messenger ribonucleic acid in rat brain by in situ hybridization. Endocrinology 121: 98–107

Seroogy KB (1986) Cholecystokinin innervation of rat forebrain. Ph. D. Dissertation, University of California, Irvine

Seroogy KB, Fallon JH (1988) Forebrain projections from cholecystokinin-like immunoreactive neurons in rat midbrain. J Comp Neurol (in press)

Seroogy KB, Mehta A, Fallon JH (1987a) Neurotensin and cholecystokinin coexistence within neurons of the ventral mesencephalon: projections to forebrain. Exp Brain Res 68: 277–289

Seroogy K, Schalling M, Chai SY, Hökfelt T, Persson H, Dixon J, Filer D, Goldstein M (1987b) Dopamine/CCK coexistence in rat ventral mesencephalon as determined by in situ hybridization histochemistry combined with immunocytochemistry. Neuroscience [Suppl] 22: S305

Seroogy K, Schalling M, Brené S, Dagerlind Å, Chai SY, Persson H, Dixon J, Filer D, Goldstein M, Walsh J, Hökfelt T (1987c) Combined in situ hybridization and immunohistochemistry for CCK and tyrosine hydroxylase in rat ventral midbrain. Soc Neurosci Abstr 13: 1088

Seroogy KB, Dangaran K, Lim S, Haycock J, Fallon JH (1988) Ventral mesencephalic neurons containing both cholecystokinin- and tyrosine hydroxylase-like immunoreactivities project to forebrain regions. J Comp Neurol (in press)

Shivers BD, Harlan RE, Pfaff DW, Schachter BS (1986) Combination of immunocytochemistry and in situ hybridization in the same tissue section of rat pituitary. J Histochem Cytochem 34: 39–43

Siegel RE, Young WS III (1985) Detection of preprocholecystokinin and preproenkephalin A mRNAs in rat brain by hybridization histochemistry using complementary RNA probes. Neuropeptides 6: 573–580

Siegel RE, Young WS III (1986) Detection of neuropeptide mRNAs by in situ hybridization histochemistry. In: Uhl GR (ed) In situ hybridization in brain. Plenum Press, New York, pp 63–71

Skirboll LR, Grace AA, Hommer DW, Rehfeld J, Goldstein M, Hökfelt T, Bunney BS (1981) Peptide-monoamine coexistence: actions of cholecystokinin-like peptide on the electrical activity of midbrain dopamine neurons. Neuroscience 11: 2111–2124

Snyder SH, Banerju SP, Yamamura HI, Greenberg D (1974) Drugs, neurotransmitters and schizophrenia. Science 184: 1243–1253

Spokes EGS (1980) Neurochemical alterations in Huntington's chorea: a study of post-mortem brain tissue. Brain 103: 179–210

Studler JM, Simon H, Cesselin F, Legrand JC, Glowinski J, Tassin JP (1981) Biochemical investigation on the cholecystokinin octapeptide in dopaminergic neurons originating from the ventral tegmental area of the rat. Neuropeptides 2: 131–139

Studler JM, Javoy-Agid F, Cesselin F, Legrand JC, Agid Y (1982) CCK-8-immunoreactivity distribution in human brain: selective decrease in the substantia nigra from parkinsonian patients. Brain Res 243: 176–179

Studler JM, Reibaud M, Tramu G, Blanc G, Glowinski J, Tassin JP (1984) Pharmacological study on the mixed CCK8/DA meso-nucleus accumbens pathway: evidence for the existence of storage sites containing the two transmitters. Brain Res 298: 91–97

Studler JM, Javoy-Agid F (1985) Cholecystokinin octapeptide immunoreactivity in human brain: modifications in parkinsonian patients. Ann NY Acad Sci 448: 656–659

Takahashi Y, Kato K, Hayashizaki Y, Wakabayashi T, Ohtsuka E, Atsuki S, Ikehara M, Matsubara K (1985) Molecular cloning of the human cholecystokinin gene by use of a synthetic probe containing deoxyinosine. Proc Natl Acad Sci USA 82: 1931–1935

Vanderhaeghen J-J, Lotstra F, De Mey J, Gilles C (1980) Immunochemical localization of cholecystokinin- and gastrin-like peptides in the brain and hypophysis of the rat. Proc Natl Acad Sci USA 77: 1190–1194

Verbanck PMP, Lotstra F, Gilles C, Linkowski P, Mendelwicz J, Vanderhaeghen J-J (1984) Reduced cholecystokinin immunoreactivity in the cerebrospinal fluid of patients with psychiatric disorders. Life Sci 34: 67–72

Wang RY, Hu X-T (1986) Does cholecystokinin potentiate dopamine action in the nucleus accumbens? Brain Res 380: 363–367

Wang RY, White FJ, Voigt MM (1984) Cholecystokinin, dopamine, and schizophrenia. Trends Pharmacol 5: 436–438

Wang RY, White FJ, Voigt MM (1985) Interactions of cholecystokinin and dopamine in the nucleus accumbens. Ann NY Acad Sci 448: 352–360

White FJ, Wang RY (1984) Interactions of cholecystokinin octapeptide and dopamine on nucleus accumbens neurons. Brain Res 300: 161–166

Whittemore SR, Ebendal T, Lärkfors L, Olson L, Seiger Å, Stromberg I, Persson H (1986) Developmental and regional expression of β-nerve growth factor messenger RNA and protein in the rat central nervous system. Proc. Natl. Acad. USA 83: 817–821

Widerlöv E, Kalivas PW, Lewis MH, Prange AJ Jr, Breese GR (1983) Influence of cholecystokinin on central monoaminergic pathways. Regul Pept 6: 99–109

Williams RG, Gayton RJ, Zhu W-Y, Dockray GJ (1981) Changes in brain cholecystokinin octapeptide following lesions of the medial forebrain bundle. Brain Res 213: 227–230

Wolfson B, Manning RW, Davis LG, Arentzen R, Baldino F Jr (1985) Colocalization of corticotropin releasing factor and vasopressin mRNA in neurons after adrenalectomy. Nature 315: 59–61

Young WS III, Bonner TI, Brann MR (1986a) Mesencephalic dopamine neurons regulate the expression of neuropeptide mRNAs in the rat forebrain. Proc Natl Acad Sci USA 83: 9827–9831

Young WS III, Mezey E, Siegel RE (1986b) Vasopressin and oxytocin mRNAs in adrenalectomized and Brattleboro rats: analysis by quantitative in situ hybridization histochemistry. Mol Brain Res 1: 231–241

Young WS III, Mezey E, Siegel RE (1986c) Quantitative in situ hybridization histochemistry reveals increased levels of corticotropin-releasing factor mRNA after adrenalectomy in rats. Neurosci Lett 70: 198–203

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