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IN SITU HYBRIDIZATION OF OXYTOCIN MESSENGER RNA: MACROSCOPIC DISTRIBUTION AND QUANTITATION IN RAT HYPOTHALAMIC CELL GROUPS

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Oxytocin mRNA was detected in the rat hypothalamus by in situ hybridization to a single stranded ^{35}S-labelled DNA probe and the distribution of oxytocin mRNA-containing cell groups was studied at the macroscopic level. Specificity of hybridization was confirmed by comparison to vasopressin mRNA hybridization in parallel tissue sections. Cell groups containing oxytocin mRNA were confined to a set of hypothalamic cell groups, i.c. the supraoptic, paraventricular, anterior commissural nuclei, nucleus circularis and scattered hypothalamic islets. These cell groups displayed similar densities of autoradiographic signals indicating that the oxytocin gene is expressed at approximately the same average level at these various sites.

In situ hybridization has recently been developed to a molecular tool to study unique mRNAs in various tissues (1,2). One of its applications in the field of neuropeptides is to locate sites of neuropeptide gene expression in the central nervous system via detection of the mRNAs coding for neuropeptide precursors. In situ hybridization has been indicated as an eminent approach for studies on the hypothalamo-neurohypophyseal system (5-10), in which the related peptides vasopressin (VP) and oxytocin (OT) are synthesized and transported by distinct neuronal cell groups (11-14). The detection of VP mRNA by in situ hybridization and its hypothalamic distribution has been reported (3-10). Here cell groups expressing the OT gene are visualized by in situ hybridization with a cloned single stranded probe. The distribution and average concentrations of OT mRNA over hypothalamic cell groups is analyzed at the macroscopic level.

Abbreviations: OT = oxytocin; VP = vasopressin.

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MATERIALS AND METHODS

Probes: The OT-specific probe was derived from the isolated rat OT gene (15) by subcloning the AvaI-BalI exon C fragment of 192 bases in a M13 vector (clone MPB-4) (16). The single stranded ^{35}S-labelled DNA probe was synthesized enzymatically using single stranded MPB-4 DNA as template and the M13 sequencing primer as primer. Label was incorporated by carrying out synthesis in the presence of 50 µCi [^{35}S,α]dCTP (1000 Ci/mmol, Amersham, UK). The probe was obtained as a single stranded fragment by restriction digestion and polyacrylamide-gel electrophoresis (16). The VP-specific probe was derived in the same way from the exon C region of the rat VP gene (16,17).

Tissue preparation: Aneasthetized male Wistar rats were perfused with 155 mM NaCl, 10 mM Na phosphate, pH 7.0 (PBS) for 5 min and with 4% (v/v) formaldehyde in PBS for 10 min. After removal from the scull, brains were incubated in the same solution for 15 min and left overnight in 15% (w/v) sucrose in PBS at room temperature. Transvers sections of 16 µm were cut in a cryostat and were thaw-mounted onto poly-L-lysine-coated slides (18).

In situ hybridization: Sections were thawed and rinced in 5 mM MgCl2 in PBS at room temperature for 10 min, then in 0.2 M Tris-HCl, 0.1 M glycine, pH 7.4 for 10 min, in 50% (v/v), 2xSSC (SSC: 150 mM NaCl, 15 mM Na citrate, pH 7.0) for 10 min and hybridized with probes ($2x10^{-4}$ dpm) in 12.5 ml per section of 50% formamide 2xSSC, 10% (w/v) dextran sulfate, 0.05% (w/v) salmon sperm DNA, 0.2% (w/v) tRNA. Hybridization was performed under coverslips in a sealed humidified box at 37 °C for 18 h. Sections were washed in 50% formamide, 2xSSC at 37 °C for 10 min, in 50% formamide, 1xSSC at 37 °C for 30 min, and finally in 1xSSC at room temperature for 30 min. Sections were dehydrated in ethanol, air dried and exposed to X-ray film (Fuji RX) for 7 days.

Densitometry: Optical densities of in situ hybridization signals on autoradiograms were determined in a video-assisted computerized image analysis system (VIPER, Gesotec, Darmstad, FRG). Optical density values were interpolated from film response curves obtained from ^{35}S-tissue standards, corrected for the background signal of the tissue section, and expressed as arbitrary units.

RESULTS

The OT-specific single stranded [^{35}S]DNA probe hybridized to various distinct sites in the rat hypothalamus and showed a different pattern of hybridization as compared to in situ hybridization with a VP-specific probe. In rostral sections the anterior commissural nucleus (ACN) was only labeled by the OT-specific probe, while the suprachiasmatic nucleus (SCN) only hybridized to the VP-specific DNA probe (not shown). In situ hybridization on serial transverse sections taken through the hypothalamus showed that the OT mRNA was located in cell groups of the supraoptic nucleus (SON), ACN, paraventricular nucleus (PVN), nucleus circularis (NC), and accessory nuclei (AN) grouped in hypothalamic islets (Fig 1). The average optical densities of hybridization signals on autoradiograms obtained from these cell groups were approximately the same (Table 1).

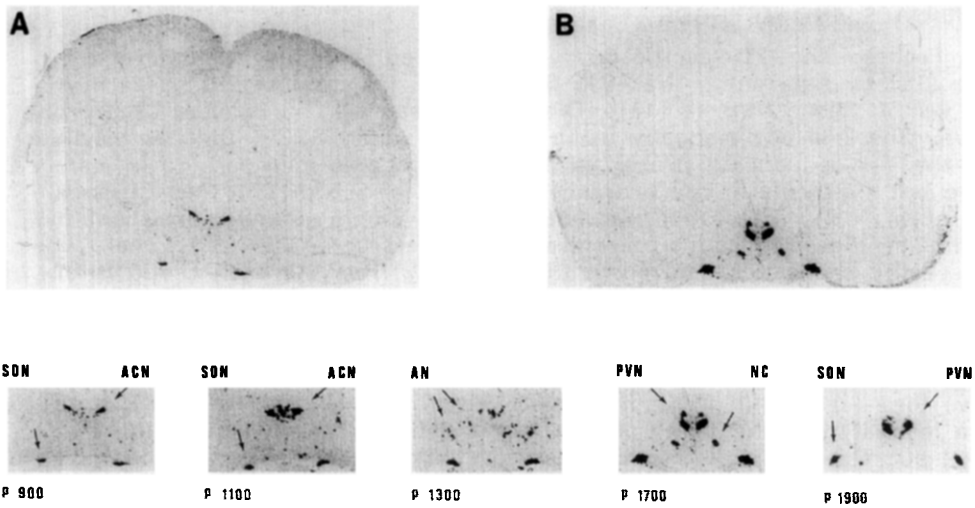

Figure 1. Detection and distribution of OT mRNA in the rat hypothalamus by in situ hybridization. The macroscopic images are shown. Whole sections are shown in upper panel A and B. The lower panel shows hybridization to OT mRNA on serial sections taken rostrally to caudally through the hypothalamus. The anatomical positions of sections are indicated by the distance in m posterior to the bregma according to Palkovits (20). Abbreviations are: SON = supraoptic nucleus, PVN = paraventricular nucleus, ACN = anterior commissural nucleus, NC = nucleus circularis, AN = accessory islets.

DISCUSSION

The results demonstrate that OT mRNA can be detected, localized and quantitated in the rat hypothalamus by in situ hybridization to a single stranded ^{35}S-labelled DNA probe in a simple protocol. The specificity of hybridization was indicated by the different patterns of hybridization with OT- and VP-specific probes. No cross-hybridization was observed between

TABLE 1

Relative concentrations of OT mRNA in hypothalamic cell groups. Values were obtained by optical density measurements of hybridization signals on autoradiograms (absorbance per square unit) standardized on a ^{35}S-autoradiography standard and expressed as arbitrary units. n is the number of observations.

Nucleus	OT mRNA concentration (arbitrary units ± S.D.)	
Supraoptic nucleus	58.7 ± 5.8	(n= 24)
Paraventricular nucleus	45.1 ± 12.7	(n= 7)
Anterior commissural nucleus	55.6 ± 8.0	(n= 13)
Nucleus circularis	58.1 ± 5.2	(n= 8)
Accessory nuclei	50.7 ± 7.7	(n= 6)

the two related mRNAs. The procedure resulted in good resolution of OT mRNA- containing cell groups on X-ray film suitable to study their distribution at the macroscopic level. Single cell resolution can also be obtained by this protocol with the use of stripping film (not shown), similar to results of others showing the presence of OT mRNA in magnocellular neurons of the SON and PVN (2,5,10). The distribution of OT mRNA-containing sites in the rat hypothalamus corresponds well with the localization of OT-containing magnocellular neurons identified by immunohistochemistry which also include the SON, PVN, ACN, NC and the hypothalamic islets (11-14). The agreement of OT mRNA hybridization and OT immunohistochemistry demonstrates the actual expression of the OT gene and active production of OT by the identified neurons.

Densitometry of OT mRNA hybridization signals from hypothalamic regions could be performed when using film sheets and provided a means to quantitate relative average OT mRNA concentrations at anatomically well defined sites. Such concentrations are dependent on the magnitude of OT gene expression, but are influenced by the anatomical organization of OT neurons within a cell group. Assuming that the density of OT neurons in these areas is not dramatically different, the measurements of in situ hybridization signals indicate that the level of expression of the OT gene in SON, PVN, ACN and NC is in the same order of magnitude. Immunohistochemical data, showing rather compact clusters of magnocellular OT-positive neurons (11-14), support this assumption. Quantitation of OT mRNA in SON and PVN by solution hybridization also points to the same conclusion (16). In situ hybridization combined with computerized densitometry provides a suitable approach to quantitate local OT mRNA concentrations with anatomical resolution, and can be applied as an extension of blotting methods to study the regulation of the OT gene in distinct hypothalamic nuclei (16,19).

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