Distribution of prodynorphin mRNA and its interaction with the NPY system in the mouse brain

Shu Lin[a], Dana Boey[a], Nicola Lee[a], Christoph Schwarzer[b], Amanda Sainsbury[a], Herbert Herzog[a,]

[a] *Neurobiology Program, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, Sydney, NSW 2010, Australia*[b] *Department of Pharmacology, Medical University of Innsbruck, Peter-Mayr-Strasse 1a, 6020 Innsbruck, Austria*

Abstract

Using radioactive in situ *hybridisation*, the distribution of prodynorphin mRNA in the brains of C57Bl/6 mice was systemically investigated, and double-labelling in situ *hybridisation* was used to determine the extent to which neuropeptide Y (NPY) and prodynorphin mRNAs were co-expressed. Our results demonstrate that prodynorphin mRNA expression in the mouse brain is localised at specific subregions of the olfactory bulb, cortex, hippocampus, amygdala, basal ganglia, thalamus, hypothalamus, mesencephalon and myelencephalon. Among the regions displaying the most intense labelling were the olfactory tubercle, lateral septum (LS), caudate putamen (Cpu), central amygdaloid nucleus (Ce), paraventricular hypothalamic nucleus (PVN), supraoptic nucleus (SO), lateral hypothalamic area (LHA), ventromedial hypothalamic nucleus (VMH), lateral reticular nucleus (LRt) and solitary tract nucleus (NTS). In the arcuate nucleus of the hypothalamus (Arc), double-labelling in situ *hybridisation* revealed that prodynorphin expressing neurons also contained NPY mRNA, with a co-localisation rate of approximately 88% in the lateral part of the Arc, and 79% in the dorsal part of the Arc, respectively, suggesting potential overlapping functions of these two neurotransmitters in feeding type behaviour.

1. Introduction

Dynorphin was first isolated from the porcine pituitary gland (Goldstein et al., 1979), was later shown to be widely expressed in the central nervous system (CNS). Dynorphin is a member of the opioid family of peptides and exerts its action through three opioid receptors, namely *kappa* (KOR), *mu* (MOR) and *delta* (DOR) (Curran and Watson, 1995), with greatest affinity for the *kappa* receptor. Several different types of peptides can be derived from the precursor peptide including dynorphin A, dynorphin B, dynorphin 32, α-neo-endorphin, and β-neo-endorphin (Rossier, 1982). Dynorphin peptides and their receptors are involved in a variety of physiological processes, including regulation of motor activity, pain modulation, inflammation response modulation, spinal nociception and energy balance (Glass et al., 1999, Palkovits, 2000 and Simonato and Romualdi, 1996).

The distribution of dynorphin in the CNS has been investigated by immunohistochemical methods in the rat (Nakao et al., 1981), hamster (Neal and Newman, 1989) and primate (Abe et al., 1988 and Khachaturian and Watson, 1982), demonstrating discrete and high levels of dynorphin peptide levels in several CNS areas. Prodynorphin mRNA expression has also been investigated in the rat brain, showing high levels of expression in the hippocampus, striatum, amygdala and hypothalamus (Morris et al., 1986, Sato et al., 1991 and Merchenthaler et al., 1997). However, there are no studies so far describing the distribution and regulation of prodynorphin mRNA in the mouse brain. This is a fundamentally important question, because more and more research on the function of dynorphin or opioid receptors makes use of knockout mice models.

Dynorphin levels are highly regulated. For example, dynorphin immunoreactivity is strongly increased in the hypothalamus of rats after food deprivation and during the dark phase, suggesting a physiological role of dynorphin and *kappa* opioid receptors in regulating food intake (Przewlocki et al., 1983). The same regulation of food intake can also be seen for another orexigenic molecule, neuropeptide Y (NPY) (Sahu et al., 1988). Interestingly, dynorphin has been shown to compete with specific binding sites for NPY on Y-receptors in vitro, suggesting that high expression of dynorphin may competitively bind to Y receptors to inhibit NPY signalling pathways (Miura et al., 1994). Also, intracerebroventricular injection of NPY or dynorphin results in a marked increase in food intake in fasted rats (Lambert et al., 1993), demonstrating that these two peptides may play a similar role in the central control of

feeding behaviour. The arcuate nucleus of the hypothalamus is a key area in regulating food intake and energy homeostasis, where both NPY and prodynorphin mRNA are reported to be expressed. This further supports a major role for these two peptides in modulating of energy balance (Baldock et al., 2002, Morris et al., 1986 and Sato et al., 1991).

In order to more directly investigate the role of hypothalamic dynorphin and NPY in the regulation of energy homeostasis, we have systematically mapped the distribution of prodynorphin mRNA expression in the mouse brain and performed co-localisation experiments to investigate whether NPY and prodynorphin mRNA expression overlap.

2. Material and methods

2.1. Animals and tissue collection

At 16 weeks of age, 18 C57Bl/6 wild type and 6 dynorphin knockout male mice were killed by cervical dislocation, and brains were removed and immediately frozen on dry ice. Six brains from wild type mice were used to map the distribution of prodynorphin mRNA and another six wild type brains were used to examine double labelling in situ *hybridisation* of NPY and prodynorphin mRNA in the hypothalamus. The remaining six wild type brains were used as controls to compare prodynorphin and NPY mRNA expression to sections from dynorphin knockout mouse brains.

2.2. In situ hybridization

Coronal slices (20 µm) of fresh frozen brains were cut and thaw-mounted on charged slides and stored at −20 °C until use. For radioactive in situ *hybridisation*, DNA oligonucleotides complementary to mouse prodynorphin gene (5′-TTCAGGACGGGTTCCA-AGAGCTTGGCATGTGCACT GATGCCT-3′) were labelled with [^{35}S] thio-dATP (Amersham Pharmacia or NEN) using terminal deoxynucleotidyltransferase (Roche, Mannheim, Germany or Amersham Pharmacia). The levels of prodynorphin mRNA expression were evaluated by measuring silver grain densities over individual neurons from photo-emulsion-dipped sections, as described previously (Sainsbury et al., 2002). The density of prodynorphin mRNA was graded as "+" (weak), "++" (moderate) or "+++" (intense).

2.3. Dual labelling in situ hybridization

Dual labelling in situ *hybridisation* was performed by combining both radioactive and non-radioactive in situ *hybridisation* protocols. For the radioactive in situ hybridisation, the mouse NPY DNA oligonucleotides (5′-GAGGGTCAGTCCACACAGCCCCATTCGCTTGTTA-CCTAGCAT- 3′) were labelled with [^{35}S] thio-dATP as described in Section 2.2. For the non-radioactive in situ hybridisation, DNA oligonucleotides specific for the prodynorphin gene were tailed with digoxigenin at the 3′ end using terminal transferase (DIG Use's Guide Manual, Boehringer Mannheim, 1995). Matching sections from the same portion of the Arc of dynorphin knockout and wild type control mice were used to examine prodynorphin mRNA expression and assayed together. The mixture of hybridisation solution consisted of 50% formamide, 5 × SSC (1 × SSC is 150 mM NaCl, 15 mM sodium citrate at pH 7.2), 500 µg/ml salmon sperm DNA, 250 µg/ml yeast tRNA, 1 × Denhardt's solution (0.02%Ficoll, 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin), 10% dextran sulfate, and 20 mM dithiothreitol. The ^{35}S-labeled probe was diluted to 10^6 cpm/ml with the hybridisation solution, and the digoxigenin-labelled probe was used at a dilution of 2.5 pmol/ml. The hybridisation solution (100 µl) was then applied to each section and incubated for 16 h at 42 °C. The sections were washed twice in 2 × SSC with 50% formamide and 100 µl of 1 M dithiothreitol at 40 °C and twice in 2 × SSC with 50% formamide at 40 °C for 15 min each, respectively. Following the washes, we used the standard colorimetric detection system using NBT and X-phosphate to detect the digoxigenin-labelling dynorphin signals (Boehringer Mannheim, Germany). Sections were air-dried and dipped in 3% parlodion (Sigma) dissolved in diethyl ether. Slides were air-dried overnight, dipped in photographic emulsion (Kodak, Rochester, New York) and then stored in foil-wrapped slide boxes at 4 °C for 2 weeks. Slides were developed with D-19 developer (Kodak, Rochester, New York), and then immersed in distilled water and cover-slipped with Aquamount$^{®}$ (Merck KGaA, Dannstadt, Germany).

2.4. Quantitation

Cell counting was performed using a grid reticule and the 10× objective of a Zeiss Axioplan light microscope (Oberkochen, Germany). Double labelled cells within the Arc were delineated through adjacent landmarks, according to the atlas of the mouse brain in stereotaxic coordinates by Franklin and Paxinos (Franklin and Paxinos, 1997). The positively double-labelled cells were counted if the number of silver grains overlying identified neuron bodies (digoxigenin-labelled) were five times higher than background levels. The values represent an average of positive neurons in a given area of one hemisphere from a single section. The average cell counts per area were determined by both left and right sides of the coronal sections, and then all groups were pooled for final analyses.

3. Results

3.1. Distribution of the prodynorphin mRNA in mouse brain

The most prominent prodynorphin mRNA expression was found in specific subregions of the cortex, olfactory bulb, hippocampus, amygdala, basal ganglia, thalamus, hypothalamus, mesencephalon and myelencephalon (Table 1). A detailed description of the specific localisation of prodynorphin mRNA is given below.

3.2. Telencephalon

In cortical regions, moderate hybridisation signals were observed over neurons in cingulate and layer 3 of the piriform cortices. Low signals were seen in primary somatosensory, agranular insular, perirhinal, lateral entorhinal and retrosplenial cortices. Neurons in the olfactory bulb and anterior olfactory nucleus exhibited weak hybridisation signals, while intensely labelled neurons were seen in the olfactory tubercle. A large number of intensely labelled neurons were also observed in the caudate putamen (Fig. 4a and b), and moderate to highly labelled neurons were found in the nucleus accumbens (core region, medial and dorsal to anterior commissure). Low to moderate hybridisation signals were expressed in the septal area, most of which were localised at the lateral septum. In the hippocampus, the granular cell layer of the dentate gyrus displayed moderate hybridisation signals while weak signals were shown in the pyramidal cell layer throughout the hippocampal proper (CA1-3) and striatum lucidem of the hippocampus.

In the amygdala, hybridisation signals were widespread over many nuclei with particular intense signals found in the medial (Fig. 4c and d) and lateral parts of cental amygdaloid nucleus. In addition, moderate signal was seen in the posterior part of medial amygdaloid and basolateral amygdaloid nuclei. In the bed nucleus of the stria terminalis, the lateral subdivisions contained weakly labelled neurons. Caudally, amygdalalohippocampal areas exhibited subpopulations of moderately labelled neurons. Weak labelling was observed in the entopeducular nucleus and ventral pallidum.

3.3. Diencephalon

In the thalamus, prodynorphin mRNA was sparsely distributed. A few neurons in the central medial thalamic and the medial habenular nuclei were moderately labelled, while small groups of neurons in the reticular nucleus and zona incerta were weakly labelled (Fig. 1).

Strong labelling was observed in a number of hypothalamic nuclei (Figs. 2 and 3). Among these, the supraoptic nucleus (Fig. 2a and b), paraventricular (Fig. 2c and d) (divisions of medial, lateral magnocellular, dorsal and posterior) and ventromedial hypothalamic nuclei (Fig. 3a and b) exhibited the most intense hybridisation signals for prodynorphin mRNA. In the medial preoptic area, moderate signals of silver grains were observed over scattered neurons, some of which were found in the region of the retrachiasmatic and anterior hypothalamic areas. High to moderate hybridisation signals were also found in the dorsomedial (Fig. 2e and f), perifornical hypothalamic nuclei, and lateral hypothalamic area (Fig. 3c and d). Moderately labelled neurons were observed in the arcuate

hypothalamic nucleus (Fig. 3e and f). In the mamillary region, weak labelling was seen in the dorsal and ventral premammillary nuclei.

Table 1
Distribution of the prodynorphin mRNA in mouse brain

Region	Hybridisation signal
Telencephalon	
Cortex	
Cingulate	+ +
Primary somatosensory	+
Agranular insular	+
Perirhinal	+
Lateral entorhinal	+
Piriform	+ +
Retrosplenial	+
Olfactory bulb	+
Anterior olfactory nucleus	+
Olfactory tubercle	+ + +
Lateral septum, ventral part	+
Nucleus accumbens	+ +
Caudate putamen (striatum)	+ + +
Hippocampus	
Dentate gyrus	+ +
CA1–CA3	+
Striatum lucidem of the hippocampus	+
Amygdaloid area	
Central amygdaloid nucleus, medial	+ + +
Central amygdaloid nucleus, lateral	+ + +
Medial amygdaloid nucleus, posterior	+ +
Basolateral amygdaloid nucleus	+ +
Bed nucleus of stria terminalis	+ +
Amygdalohippocampal area	+ +
Entopedicular nucleus	+
Ventral pallidum	+
Diencephalon	
Thalamus	
Central medial thalamic nucleus	+
Reticular thalamic nucleus	+
Medial habenular	+ +
Zona incerta	+
Hypothalamus	
Medial preoptic nucleus	+ +
Retrachiasmatic area	+ +
Supraoptic nucleus	+ + +
Anterior hypothalamic area	+ +
Paraventricular nucleus	
Periventricular	+
Posterior subdivisions	+ + +
Medial magnocellular subdivision	+ + +
Lateral magnocellular subdivision	+ + +
Dorsomedial nucleus	+ + +
Lateral hypothalamus	+ +
Perifornical nucleus	+ +
Ventromedial nucleus	+ + +
Arcuate nucleus	+ +
Mesencephalon and mylencephalon	
Inferior colliculus	+
Dorsal raphe nucleus	+
Peripeduncular nucleus	+
Periaqueductal gray	+
Substantia nigra, lateral part	+
Lateral Parabrachial nucleus	+
Solitary tract nucleus	+ + +
Lateral reticular nucleus	+ + +

The level of signal is a representation of relative density of pre-prodynorphin mRNA over individual cell groups. The density of signals was graded as "+" (weak), "++" (moderate) or "+++" (intense).
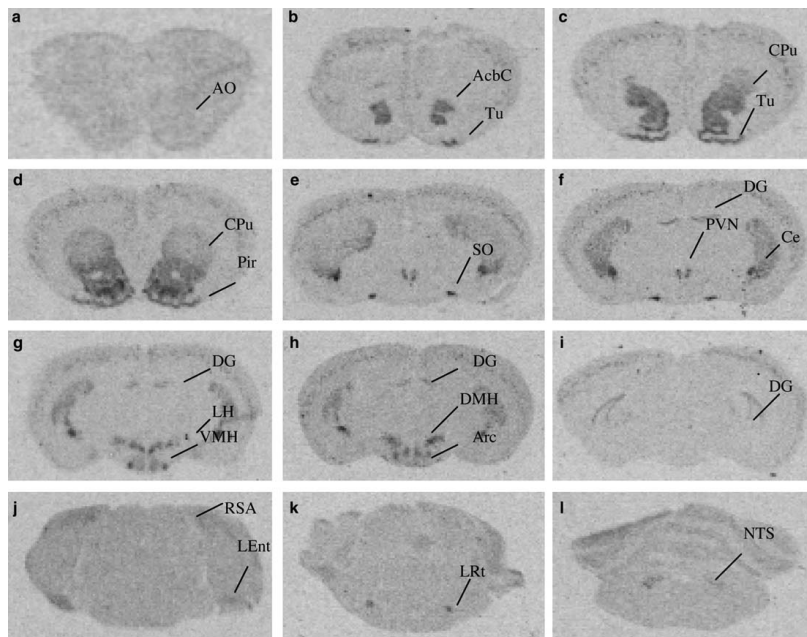
Fig. 1. Autoradiograms of prodynorphin mRNA expression in the mouse. The slides containing coronal mouse brain sections are presented in rostrocaudal sequence. Some major neuroanatomical landmarks are indicated on the right side of each autoradiogram. *Abbreviations:* AO, anterior olfactory nucleus; AcbC, nucleus accumbens; Arc, accurate hypothalamic nucleus; Ce, central amygdaloid nucleus; Cpu, caudate/putamen; DG, dentate gyrus; DMH, dorsomedial hypothalamic nucleus; LEnt, lateral entorhinal cortex; LH, lateral hypothalamic area; LRt, lateral reticular nucleus; NTS, nucleus of the solitary tract; Pir, piriform cortex; PVN, paraventricular hypothalamic nucleus; RSA, retrosplential cortex; SO, supraoptic nucleus; Tu, olfactory tubercle; VMH, ventromedial hypothalamic nucleus. Magnification, 10×.
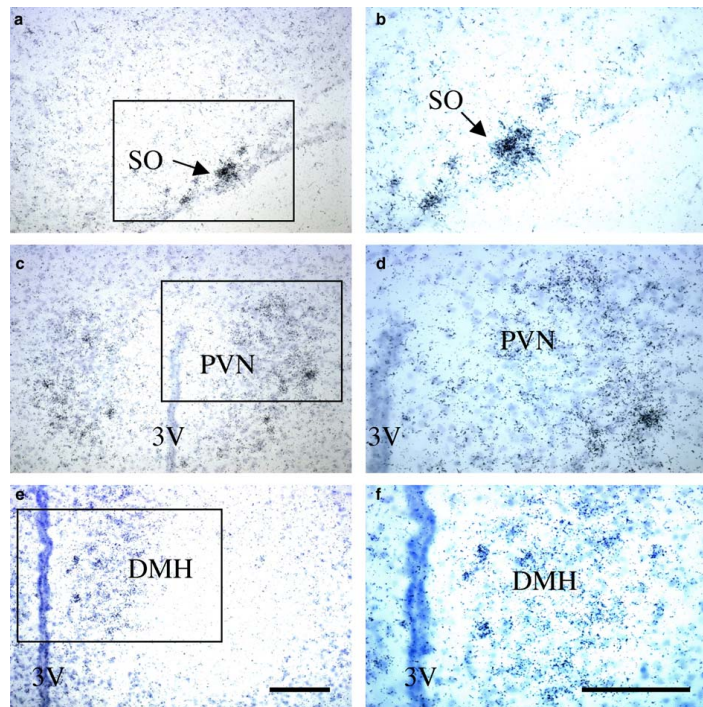

Fig. 2. Emulsion-dipped autoradiographs of prodynorphin mRNA expression of in the hypothalamus. (a),(c) and (e): brightfield low-magnification micrograph of the supraoptic nucleus, paraventricular hypothalamic nucleus and dorsomedial hypothalamic nucleus, respectively. (b),(d) and (f): a high-magnification of micrograph of the supraoptic nucleus, paraventricular hypothalamic nucleus and dorsomedial hypothalamic nucleus, respectively; 3 V, the third ventricle; SO, supraoptic nucleus; PVN, paraventricular hypothalamic nucleus; DMH, dorsomedial hypothalamic nucleus; Scale bars = 40 μm in (a),(c) and (e), and 25 μm in (b),(d) and (f).
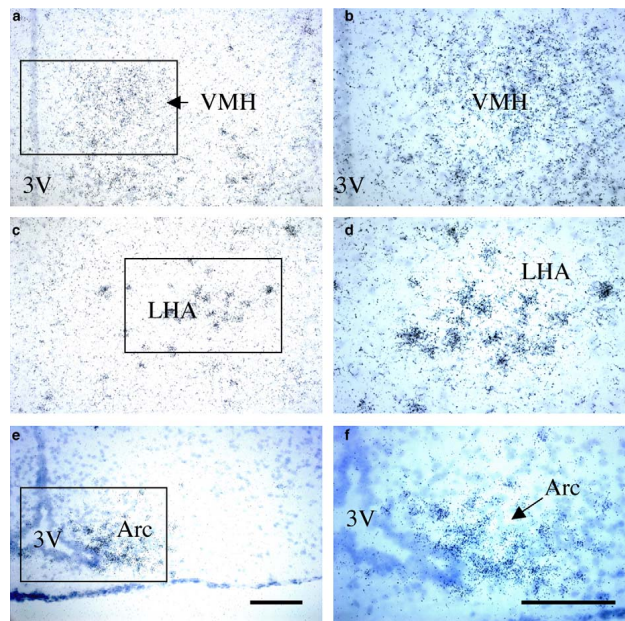
Fig. 3. Emulsion-dipped autoradiographs of the expression of prodynorphin mRNA in the hypothalamus. (a),(c) and (e): brightfield low-magnification micrograph of the ventromedial hypothalamic nucleus, lateral hypothalamic area and arcuate hypothalamic nucleus. (b),(d) and (f): a high-magnification of micrograph of the ventromedial hypothalamic nucleus, lateral hypothalamic area and arcuate hypothalamic nucleus; 3 V, the third ventricle. VMH, ventromedial hypothalamic nucleus; LHA, lateral hypothalamic area; Arc, accurate hypothalamic nucleus; Scale bars = 40 μm in (a),(c) and (e), and 25 μm in (b),(d) and (f).

3.4. Mesencephalon and mylencephalon

Few regions of the midbrain and hindbrain were shown to contain significant levels of prodynorphin mRNA (Table 1, Fig. 4). Sporadic positive neurons were evident in the inferior colliculus, dorsal raphe nucleus, peripeduncular nucleus and periaqueductal gray. The lateral part of substantia nigra and parabrachial nucleus were observed only a very scattered prodynorphin mRNA. The solitary tract nucleus (Fig. 4e and f) and lateral reticular nucleus contained intensely labelled cells.
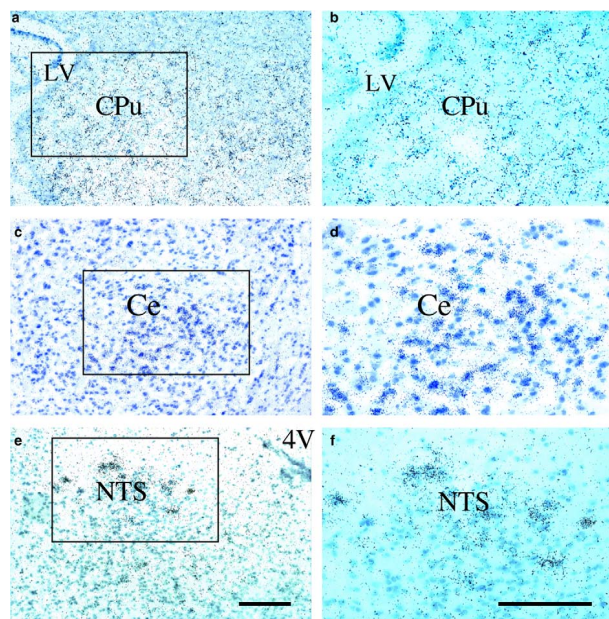

Fig. 4. Emulsion-dipped autoradiographs of the expression of prodynorphin mRNA in the caudate/putamen, central amygdaloid nucleus and nucleus of the solitary tract. (a),(c) and (e): low-magnification micrograph of the caudate/putamen, central amygdaloid nucleus and nucleus of the solitary tract. (b),(d) and (f): a high-magnification of micrograph of the caudate/putamen, central amygdaloid nucleus and nucleus of the solitary tract; LV, the lateral ventricle; 4 V, the fourth ventricle; Ce, central amygdaloid nucleus; Cpu, caudate/putamen; NTS, nucleus of the solitary tract; Scale bars = 40 μm in (a),(c) and (e), and 25 μm in (b),(d) and (f).

3.5. Co-localisation of NPY and prodynorphin mRNA in the hypothalamus

In order to investigate the possible interaction of dynorphin with the NPY system we performed double-labelling experiments employing NPY ^{35}S-labelled and prodynorphin-digoxigenin-labelled probes. The largest numbers of co-expressing neurons were found in the Arc (Fig. 5c and d). NPY mRNA was co-expressed in approximately 80% of Arc dynorphin neurons (79% located in the dorsal part of Arc and 88% located in lateral part of Arc). No co-localisation of NPY and dynorphin could be detected in any other hypothalamic nuclei (data not shown), however, several other parts of the brain including the central amygdala (approx 30%), the posterior part of the medial amygdala (approx 25%) and the caudata putamen (approx 25%) show co-localisation of NPY and dynorphin mRNA. As a control, no prodynorphin mRNA expression was shown in the Arc of dynorphin knockout mice (Fig. 5b). Interestingly, the NPY mRNA expression level in the arc of dynorphin knockout mice was reduced by approximately 26% (Fig. 6) further suggesting a close interaction between these two neurotransmitter systems.
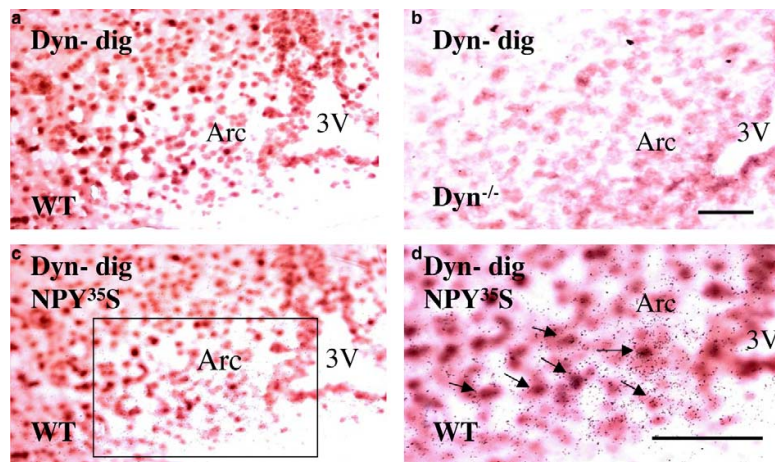

Fig. 5. Brightfield micrographs showing neurons that co-express NPY and prodynorphin mRNA in the arcuate hypothalamic nucleus of the mouse. (a): digoxigenin reaction product showing prodynorphin mRNA expression in wild type mice. (b): control showing no prodynorphin mRNA expression in the Arc of dynorphin knockout mice. (c): low-magnification micrograph shows neurons co-expressing neuropeptide Y and prodynorphin mRNA in the arcuate hypothalamic nucleus of wild type mice. (d): (c) boxed area is shown in high-magnification. Arrows indicate double-labelled neurones. WT, wild type mice; Dyn$^{-/-}$, dynorphin knockout mice; Arc, arcuate hypothalamic nucleus; 3 V, the third ventricle; Dyn-dig, prodynorphin labelled with digoxigenin; NPY^{35}S, NPY labelled with [^{35}S]; Scale bars = 40 μm in (a),(b) and (c), and 25 μm in (d).
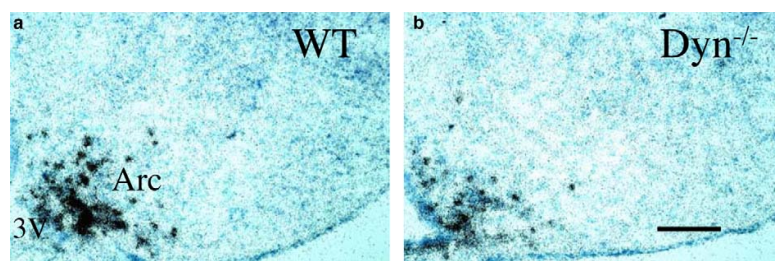

Fig. 6. Emulsion-dipped autoradiographs of the expression of NPY mRNA in the arcuate hypothalamic nucleus of (a): wild type and (b): dynorphin knockout mice. 3 V, the third ventricle; Arc, accurate hypothalamic nucleus; WT, wild type mice; Dyn$^{-/-}$, dynorphin knockout mice; Scale bars = 40 μm.

4. Discussion

This study shows for the first time that Arc NPY neurons contain prodynorphin mRNA. Previously, it has been known that peripherally administrated leptin induces c-fos expression in dynorphin positive neurons in the Arc, suggesting that like NPY, dynorphin is also regulated by leptin (Elias et al., 2000). This is supported by the fact that after food deprivation, both NPY and prodynorphin mRNA are significant increased in the hypothalamus (Przewlocki et al., 1983 and Sahu et al., 1988). Furthermore, prodynorphin mRNA expression

is increased five-fold in the DMN of leptin deficient *ob/ob* mice (Khawaja et al., 1991), the DMN being a region that shows intense dynorphin expression in the mouse. Therefore, it is possible that dynorphin and NPY interact within the hypothalamus in the regulation of food intake and energy homeostasis.

The present study also demonstrates a conserved expression pattern for the prodynorphin mRNA in the mouse brain compared to rats. The regions that exhibited highest hybridisation signals for prodynorphin mRNA include the olfactory tubercle, caudate putamen, sub-group of the amygdala, most of the hypothalamic nuclei, and several brain stem nuclei. However, there were also differences found between the prodynorphin mRNA expression in the mouse to that of the rat, particularly in the central amygdaloid and the lateral reticular nucleus which displayed intense signals in the mouse, but only low to moderate signals in the rat (Merchenthaler et al., 1997).

Overall our results are consistent with immunohistochemistry studies investigating the distribution of dynorphin neurons in the rat brain (Fallon and Leslie, 1986). In the striatal region, we found the strongest prodynorphin mRNA expression in the olfactory tubercle, caudate putamen and nucleus accumbens. Previous studies show that prodynorphin mRNA is expressed in GABAergic neurons in the stratum, projecting to the substantia nigra and pallidum (Graybiel, 1986), and that dynorphin gene expression is controlled by the dopaminergic (Morris et al., 1988) and serotoninergic systems (Morris et al., 1988). The nucleus accumbens receive inputs mainly from the limbic system, which then sends outputs to the ventral pallidum and ventral tegmental area. Strong dynorphin signal was displayed in the striatal region, suggesting that dynorphin may play a role in the regulation of adaptive behavioural response, stress, drug abuse, obsessive compulsive disorders, seizures and psychoses (Laughlin et al., 2001, Shippenberg and Rea, 1997, Shirayama et al., 2004 and Simonato and Romualdi, 1996).

Our results show high levels of prodynorphin mRNA expression in the dentate gyrus of the mouse brain, which supports previous reports that dynorphin-derived peptides are synthesised in the granule cells of the dentate gyrus in rats, which is then transported to the axon terminals in the C3 region of the hippocampus (Morris and Johnston, 1995). High levels of dynorphin in the dentate gyrus have been suggested to be involved in seizures as well as addictive drug and alcohol-seeking behaviour (Cowen et al., 1998, Morris and Johnston, 1995 and Romualdi et al., 1991). In the amygdala area, there are intense to moderate hybridisation signals in several subregions such as the central amygdala, bed nucleus of stria terminals and basolateral amygdaloid nucleus suggesting an involvement of dynorphin in mood disorders, depression and stress-related changes in pain sensitivity (Hurd, 2002 and Schulz et al., 1996). Here we also show that there are high level of prodynorphin mRNA expression in the NTS and lateral reticular nucleus, which provide both ascending innervations to the hypothalamus and descending projections to the spinal cord. Both of these nuclei are also strategically located in brain regions that have long been associated with the regulation of autonomic functions such as the cardiovascular, respiratory and nociceptive control mechanisms.

In addition, strong prodynorphin mRNA expression is found in the PVN and SO. In the PVN, intense prodynorphin mRNA expression is shown in the posterior PVN as well as the medial and lateral magnocellular part of the PVN. Co-localisation of dynorphin and oxytocin or vasopressin neurons in the magnocellular neurons of the PVN and SO has been reported (Meister et al., 1990), suggesting an important role for dynorphin in the control of prolactin secretion and water homeostasis. In addition, colocalisation of prodynorphin with two other neuropeptides (Cocaine and amphetamine regulated transcript and Orexin), which play an important role in the regulation of feeding behaviour, has been shown in the PVN and SON (Elias et al., 2001), as well as in the parvicellular neurons in the LH (Smart and Jerman, 2002).

We also demonstrate co-localisation of prodynorphin mRNA with NPY in several brain areas. Whereas co-expression in different parts of the amygdala indicate overlapping or coordinated function in anxiety-related behaviours the highest extend of overlap of NPY and dynorphin is found in neurons of the Arc. This supports a potential coordinated function of dynorphin and NPY in the modulation of energy homeostasis and neuroendocrine regulation. Further evidence for this comes from the observed down regulation of NPY mRNA in dynorphin knockout mice.

In summary, the present study provides a precise map of prodynorphin mRNA expression and co-expression with NPY in the mouse brain, which will facilitate interpretation and design of dynorphin or opioid receptors knockout and conditional knockout studies in mice.

References

Abe, J., Okamura, H., Kitamura, T., Ibata, Y., Minamino, N., Matsuo, H., Paull, W.K., 1988. Immunocytochemical demonstration of dynorphin(PH-8 P)-like immunoreactive elements in the human hypothalamus. Journal of Comparative Neurology 276, 508.

Baldock, P.A., Sainsbury, A., Couzens, M., Enriquez, R.F., Thomas, G.P., Gardiner, E.M., Herzog, H., 2002. Hypothalamic Y2 receptors regulate bone formation. Journal of Clinical Investigation 109, 915.

Cowen, M.S., Rezvani, A., Jarrott, B., Lawrence, A.J., 1998. Distribution of opioid peptide gene expression in the limbic system of Fawn-Hooded (alcohol-preferring) and Wistar-Kyoto (alcohol- non-preferring) rats. Brain Research 796, 323.

Curran, E.J., Watson Jr., S.J., 1995. Dopamine receptor mRNA expression patterns by opioid peptide cells in the nucleus accum- bens of the rat: A double in situ hybridization study. Journal of Comparative Neurology 361, 57.

Elias, C.F., Kelly, J.F., Lee, C.E., Ahima, R.S., Drucker, D.J., Saper, C.B., Elmquist, J.K., 2000. Chemical characterization of leptin- activated neurons in the rat brain. Journal of Comparative Neurology 423, 261.

Elias, C.F., Lee, C.E., Kelly, J.F., Ahima, R.S., Kuhar, M., Saper, C.B., Elmquist, J.K., 2001. Characterization of CART neurons in the rat and human hypothalamus. Journal of Comparative Neurology 432, 1.

Fallon, J.H., Leslie, F.M., 1986. Distribution of dynorphin and enkephalin peptides in the rat brain. Journal of Comparative Neurology 249, 293.

Franklin, K.B., Paxinos, G., 1997. The Mouse Brain in Stereotaxic Coordinates. Academic Press, San Diego.

Glass, M.J., Billington, C.J., Levine, A.S., 1999. Opioids and food intake: Distributed functional neural pathways? Neuropeptides 33, 360.

Goldstein, A., Tachibana, S., Lowney, L.I., Hunkapiller, M., Hood, L., 1979. Dynorphin-(1-13), an extraordinarily potent opioid peptide. Proceedings of the National Academy of Sciences of the United States of America 76, 6666.

Graybiel, A.M., 1986. Neuropeptides in the basal ganglia. Research Publications – Association for Research in Nervous and Mental Disease 64, 135.

Hurd, Y.L., 2002. Subjects with major depression or bipolar disorder show reduction of prodynorphin mRNA expression in discrete nuclei of the amygdaloid complex. Molecular Psychiatry 7, 75.

Khachaturian, H., Watson, S.J., 1982. Some perspectives on mono- amine-opioid peptide interaction in rat central nervous system. Brain Research Bulletin 9, 441.

Khawaja, X.Z., Chattopadhyay, A.K., Green, I.C., 1991. Increased beta-endorphin and dynorphin concentrations in discrete hypothalamic regions of genetically obese (ob/ob) mice. Brain Research 555, 164.

Lambert, P.D., Wilding, J.P., al-Dokhayel, A.A., Bohuon, C., Comoy, E., Gilbey, S.G., Bloom,

S.R., 1993. A role for neuropeptide-Y, dynorphin, and noradrenaline in the central control of food intake after food deprivation. Endocrinology 133, 29.

Laughlin, T.M., Larson, A.A., Wilcox, G.L., 2001. Mechanisms of induction of persistent nociception by dynorphin. Journal of Pharmacology and Experimental Therapeutics 299, 6.

Meister, B., Villar, M.J., Ceccatelli, S., Hokfelt, T., 1990. Localization of chemical messengers in magnocellular neurons of the hypothalamic supraoptic and paraventricular nuclei: An immunohisto- chemical study using experimental manipulations. Neuroscience 37, 603.

Merchenthaler, I., Maderdrut, J.L., Cianchetta, P., Shughrue, P., Bronstein, D., 1997. In situ hybridization histochemical localiza- tion of prodynorphin messenger RNA in the central nervous system of the rat. Journal of Comparative Neurology 384, 211.

Miura, M., Inui, A., Sano, K., Ueno, N., Teranishi, A., Hirosue, Y., Nakajima, M., Okita, M., Togami, J., Koshiya, K., et al., 1994. Dynorphin binds to neuropeptide Y and peptide YY receptors in human neuroblastoma cell lines. American Journal of Physiology 267, E702.

Morris, B.J., Haarmann, I., Kempter, B., Hollt, V., Herz, A., 1986. Localization of prodynorphin messenger rna in rat brain by in situ hybridization using a synthetic oligonucleotide probe. Neuroscience Letters 69, 104.

Morris, B.J., Hollt, V., Herz, A., 1988. Dopaminergic regulation of striatal proenkephalin mRNA and prodynorphin mRNA: Contrasting effects of D1 and D2 antagonists. Neuroscience 25, 525.

Morris, B.J., Johnston, H.M., 1995. A role for hippocampal opioids in long-term functional plasticity. Trends in Neuroscience 18, 350.

Nakao, K., Yoshimasa, T., Oki, S., Tanaka, I., Nakai, Y., Wakimasu, M., Fujino, M., Imura, H., 1981. Presence of dynorphin-like immunoreactivity in rat pituitary gland and hypothalamus. Regulatory Peptides 2, 201.

Neal Jr., C.R., Newman, S.W., 1989. Prodynorphin peptide distribution in the forebrain of the Syrian hamster and rat: A comparative study with antisera against dynorphin A, dynorphin B, and the C- terminus of the prodynorphin precursor molecule. Journal of Comparative Neurology 288, 353.

Palkovits, M., 2000. Stress-induced expression of co-localized neuropeptides in hypothalamic and amygdaloid neurons. European Journal of Pharmacology 405, 161.

Przewlocki, R., Lason, W., Konecka, A.M., Gramsch, C., Herz, A., Reid, L.D., 1983. The opioid peptide dynorphin, circadian rhythms, and starvation. Science 219, 71.

Romualdi, P., Lesa, G., Ferri, S., 1991. Chronic opiate agonists down- regulate prodynorphin gene expression in rat brain. Brain Research 563, 132.

Rossier, J., 1982. Opioid peptides have found their roots. Nature 298, 221.

Sahu, A., Kalra, P.S., Kalra, S.P., 1988. Food deprivation and ingestion induce reciprocal changes in neuropeptide Y concentrations in the paraventricular nucleus. Peptides 9, 83.

Sainsbury, A., Schwarzer, C., Couzens, M., Herzog, H., 2002. Y2 receptor deletion attenuates the type 2 diabetic syndrome of ob/ob mice. Diabetes 51, 3420.

Sato, M., Morita, Y., Saika, T., Fujita, M., Ohhata, K., Tohyama, M., 1991. Localization and ontogeny of cells expressing preprodynor- phin mRNA in the rat cerebral cortex. Brain Research 541, 41.

Schulz, S., Schreff, M., Nuss, D., Gramsch, C., Hollt, V., 1996. Nociceptin/orphanin FQ and opioid peptides show overlapping distribution but not co-localization in pain modulatory brain regions. Neuroreport 7, 3021.

Shippenberg, T.S., Rea, W., 1997. Sensitization to the behavioral effects of cocaine: modulation by dynorphin and kappa-opioid receptor agonists. Pharmacology Biochemistry and Behavior 57, 449.

Shirayama, Y., Ishida, H., Iwata, M., Hazama, G.I., Kawahara, R., Duman, R.S., 2004. Stress increases dynorphin immunoreactivity in limbic brain regions and dynorphin antagonism produces antidepressant-like effects. Journal of Neurochemistry 90, 1258.

Simonato, M., Romualdi, P., 1996. Dynorphin and epilepsy. Progress in Neurobiology 50, 557.

Smart, D., Jerman, J., 2002. The physiology and pharmacology of the orexins. Pharmacology and Therapeutics 94, 51.