Localization of Constitutive and Hyperthermia-Inducible Heat Shock mRNAs (hsc70 and hsp70) in the Rabbit Cerebellum and Brainstem by Non-Radioactive In Situ Hybridization

J.A. Foster, S.J. Rush, and I.R. Brown

Department of Zoology, University of Toronto, West Hill, Ontario, Canada

Neural expression of constitutive hsc70 mRNA and hyperthermia-inducible hsp70 mRNA is examined using radioactive and non-radioactive in situ hybridization procedures. A strong induction of hsp70 mRNA was noted in cell populations in cerebellar layers and in the brainstem which demonstrated expression of mRNA encoding proteolipid protein, an oligodendrocyte marker. The non-radioactive in situ hybridization procedure using digoxigenin (DIG)-UTP-labeled riboprobes permitted improved signal localization, and stress-inducible hsp70 mRNA was detected at the cytoplasmic cap areas of individual oligodendrocytes. Cell types which express constitutive members of the hsc/hsp70 multigene family were also identified. Neurons in the brainstem and in the deep white matter and molecular layer of the cerebellum showed expression of hsc70 mRNA while signal was not detected in adjacent glial cells. A neuron-specific enolase riboprobe aided in the identification of neuronal cell types. The non-radioactive DIG riboprobe revealed that hsc70 mRNA was highly localized to the cytoplasm of individual neurons. High constitutive levels of hsc70 in certain neurons may dampen hsp70 induction after hyperthermia in these cell populations. © 1995 Wiley-Liss, Inc.

Key words: neurons, oligodendrocytes, stress proteins

INTRODUCTION

In response to stresses, such as elevated temperature, cells increase synthesis of a group of highly conserved proteins while decreasing synthesis of most cellular proteins. This well-established phenomenon is known as the heat shock response (for review, see Nover, 1991; Georgopoulos and Welch, 1993). Heat shock proteins (hsps) are found in all cells and are clas-

sified into families based on their molecular weight (Lindquist and Craig, 1988). The most extensively studied is the hsp70 family (for review, see Morimoto et al., 1990). Hsp70s are a multigene family which comprises both constitutively expressed members and stress-inducible members. The research presented here focuses on the expression of constitutive hsc70 mRNA and hyperthermia-inducible hsp70 mRNA in the rabbit central nervous system.

Constitutively expressed hsc70 members have been found to be involved in normal cellular function (Welch et al., 1989). Protein-protein interactions including protein folding, assembly, and translocation involve hsc70 protein (Chirico et al., 1988; Deshaies et al., 1988; Beckmann et al., 1990; Gething and Sambrook, 1992). Within the nervous system, hsc70 is involved in the uncoating of triskelions of clathrin-coated vesicles (Ungewickell, 1985; Chappell et al., 1986; Black et al., 1991). Following neural traumas such as hyperthermia, ischemia, or wounding, hsp70 is induced (for review, see Brown, 1990, 1994). In the stressed cell, hsp70 protein is thought to play a role in cellular protection and repair (Pelham, 1986, 1988; Craig, 1989). Studies have shown that impairment of hsp70 induction leads to thermosensitive cells (Johnston and Kucey, 1988; Riabowol et al., 1988), and that sublethal exposures to heat can confer thermotolerance (for review, see Nover, 1991). Similarly, within the nervous system, recent experiments, at both the in vivo and in vitro level, have demonstrated that prior heat shock can have neuroprotective effects on subsequent insults (Walsh et al., 1987, 1989, 1994; Barbe et al., 1988; Lowenstein et al., 1991; Rordorf et al., 1991).

Received August 8, 1994; revised October 14, 1994; accepted October 17, 1994.

Address reprint requests to I.R. Brown, Dept. of Zoology, Univ. of Toronto, Scarborough Campus, 1265 Military Trail, West Hill, Ontario, M1C 1A4 Canada.

Previous studies in our laboratory have demonstrated that an increase in body temperature of 2–3°C results in a strong induction of hsp70 in fibre tracts of the forebrain, cerebellum, and spinal cord (Sprang and Brown, 1987; Manzerra and Brown, 1992a,b). Within the Purkinje layer of the cerebellum, our studies have shown constitutive expression of hsc70 mRNA and protein in Purkinje neurons and in 1-hour hyperthermic animals, an induction of hsp70 mRNA and protein in Bergmann glial cells, a specialized type of astrocyte (Manzerra et al., 1993).

In the present investigation we focus on a second glial cell type, the oligodendrocyte, and on additional neuronal populations. We examine hsc70 and hsp70 mRNA expression in the deep white matter and molecular layer of the cerebellum and in the brainstem. To aid in the identification of the different neural cell types, two criteria were used, morphology and expression of marker mRNA species. Neuron-specific enolase (nse) was used as a neuronal marker and proteolipid protein (plp) was used as an oligodendrocyte marker. In situ hybridization is utilized to identify neural cell types which are expressing constitutive and stress-inducible members of the hsc/hsp70 multigene family. In addition to the use of radioactive riboprobes, a non-radioactive in situ hybridization protocol employing digoxigenin (DIG)-UTP labeled riboprobes and antibody detection of signal was adopted since the procedure is rapid and improves the ability to localize signal to individual cells in the nervous system.

MATERIALS AND METHODS

Treatment of Animals

The body temperature of adult male New Zealand white rabbits (mean weight = 1.8 kg $\pm$ 0.1, n = 6) was elevated approximately 3°C above normal (39.6°C) by the intravenous injection of LSD at 100 μ g/kg as previously described (Cosgrove and Brown, 1983). We have shown that the induction of hsp70 mRNA in the rabbit brain is due to hyperthermic effects of this drug (Manzerra and Brown, 1990). The core body temperature of the animals was monitored, prior to injection and at 15-minute intervals, with a rectal thermistor probe. The core body temperature reached maximum temperature at 1 hour post-LSD injection, at which time animals were sacrificed.

Synthesis of Riboprobes

The subclone pH 2.3, a 2.3-kb HindIII-BamHI fragment of the human hsp70-inducible gene in pGEM1, was obtained from R. Morimoto (Wu et al., 1985). An 843-bp insert was digested out with ClaI and HindIII and subcloned into the vector pGEM1 for preparation of ³⁵S

or ³²P-labeled antisense riboprobes, and into the vector pSP72 for preparation of ³⁵S or ³²P-labeled sense riboprobes. Linearization with ClaI and HindIII and subsequent in vitro transcription using T7 and SP6 polymerases, respectively, produced antisense and sense hsp70 riboprobes. The ClaI/HindIII insert was subcloned into the vector pBluescript KS for preparation of digoxigenin (DIG) hsp70 riboprobes using a DIG system supplied by Boehringer Mannheim. Linearization with XhoI and HindIII and subsequent in vitro transcription using T7 and T3 polymerases produced antisense and sense DIG-hsp70 riboprobes, respectively.

The subclone pH 7.6, a 600 bp EcoRI fragment from the human constitutive hsc70-related protein p72 in pGEM2 was obtained from R. Morimoto (Watowich and Morimoto, 1988). Production of antisense and sense ³⁵S/³²P-hsc70 riboprobes was achieved by linearization with HindIII and SphI, followed by in vitro transcription by SP6 and T7 polymerases, respectively. The 600-bp EcoRI fragment was digested out and subcloned into the vector pBluescript KS for preparation of DIG-hsc70 riboprobes. Linearization with HindIII and BamHI and subsequent in vitro transcription using T7 and T3 polymerases produced antisense and sense DIG-hsc70 riboprobes, respectively.

The subclone pCD169, a 2,270 bp cDNA encoding neuron-specific enolase (nse), was obtained from J. Gregor Sutcliffe (Forss-Petter et al., 1986). A 563-bp insert was digested out with PstI and HindIII and subcloned into the vector pSP72 for preparation of ³⁵S or ³²P-labeled nse riboprobes. The plasmid was linearized with PstI and HindIII and transcribed with SP6 and T7 polymerases, respectively, to generate anti-sense and sense nse riboprobes.

The subclone p20, a 1,430 bp sequence of proteolipid protein pUC18, was obtained from J. Gregor Sutcliffe (Milner et al., 1985). A 850-bp insert was digested out with BamHI and PstI and subcloned into pGEM4 for preparation of ³⁵S or ³²P-labeled plp riboprobes. The plasmid was linearized with BamHI and SmaI, transcribed with T7 and SP6 polymerases to generate anti-sense and sense plp riboprobes, respectively.

Northern Blot Analysis

Total RNA was isolated from control and heat shock cerebellum and brainstem, cerebral hemispheres, and kidney. RNA samples were run on a formaldehyde/agarose gel (5 μ g RNA/lane), blotted overnight to a nylon membrane, and fixed under ultraviolet light. Parallel blots were hybridized with ³²P-labeled hsc70, hsp70, neuron-specific enolase (nse), and proteolipid protein (plp) riboprobes. Equal loading was determined by methylene blue staining.

Fig. 1. Northern blot analysis of riboprobes. Total RNA was isolated from control (C) and heat shock (HS) cerebellum plus brainstem (CB), cerebral hemispheres (CH), and kidney (KID) and subjected to Northern blot analysis. Parallel Northern blots (5 μ g RNA/lane) were hybridized with 32 P-labeled hsc70, hsp70, nse, and plp riboprobes. The size of the RNA transcripts detected is indicated in kilobases (kb). Equal loading was determined by methylene blue staining.

Tissue Preparation

Animals were anaesthetized with pentobarbital (80 mg/kg) injected via the marginal ear vein and perfused intracardially with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in PBS. Cerebellum and brainstem were removed, postfixed in 4% paraformaldehyde overnight, equilibrated in an increasing series of phosphate-buffered sucrose, frozen in 2-methylbutane (-30°C to -40°C), and stored at -70°C until use. A series of 10- μ m frozen sections was collected on gelatin-coated glass slides (1% gelatin, 0.5% chromium potassium sulphate) and air dried overnight.

Pretreatment of Sections

Tissue sections were rehydrated in PBS for 30 minutes. To facilitate probe penetration, sections were incu-

bated in protease (5.0 μ g/ml in 100 mM Tris, pH 8.0, 50 mM EDTA) for 20 minutes at 37°C , washed for 10 minutes in 0.1 M triethanolamine, pH 8.0, and dehydrated through an ethanol series containing 0.33 M ammonium acetate.

Radioactive In Situ Hybridization

Sections were prehybridized for 2 hours at 42°C in hybridization buffer containing 50% formamide, 750 mM NaCl, 0.1% Ficoll 0.1%, polyvinylpyrrolidone, 0.1% bovine serum albumin, 0.1% sodium dodecyl sulphate, 100 mM dithiothreitol, and 50 mM PIPES, pH 7.4. Tissue sections were hybridized overnight, at 55°C , with 40 μ l of hybridization buffer containing 2×10^6 cpm of one of the ^{35}S -UTP-labeled riboprobes: hsc70 antisense or sense riboprobes, hsp70 antisense or sense riboprobes, nse antisense or sense riboprobes, or oligodendrocyte-specific plp antisense or sense riboprobes. Tissue sections were hybridized with either the hsc70 or the hsp70 riboprobes and alternate sections with the nse or plp riboprobes. Slides were rinsed in Tris/NaCl buffer (500 mM NaCl, 1 mM EDTA, 10 mM Tris, pH 8.0), incubated for 30 minutes in RNase buffer (500 mM NaCl, 1 mM EDTA, 10 mM Tris, pH 7.5) containing 20 μ g/ml RNase A, and washed for 1 hour at 37°C in RNase buffer containing 0.3 M β -mercaptoethanol followed by 1 hour wash at 70°C in $0.1 \times \text{SSC}$, 0.3 M β -mercaptoethanol. The slides were dehydrated through an ethanol series containing 0.33 M ammonium acetate, air dried, and processed for autoradiography with NTB2 Kodak liquid emulsion.

Non-Radioactive In Situ Hybridization

Sections were prehybridized for 2 hours at 42°C in hybridization buffer containing 50% formamide, 0.1 M Tris, 50 mM PIPES, pH 7.4, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin, 0.1% sodium dodecyl sulphate, and 0.05% n-lauroylsarcosine. Tissue sections were hybridized overnight, at 55°C , with 40 μ l of hybridization buffer containing 100 ng of one of the digoxigenin-UTP-labeled heat shock riboprobes: hsc70 antisense or sense riboprobes or hsp70 antisense or sense riboprobes. Alternate sections of a series were hybridized with either the hsc70 or the hsp70 riboprobes. Slides were rinsed in Tris/NaCl buffer (500 mM NaCl, 1 mM EDTA, 10 mM Tris, pH 8.0), incubated for 30 minutes in RNase buffer (500 mM NaCl, 1 mM EDTA, 10 mM Tris, pH 7.5) containing 20 μ g/ml RNase A, and washed for 1 hour at 37°C in RNase buffer followed by a 1-hour wash at 60°C in $0.1 \times \text{SSC}$. The slides were processed using alkaline phosphatase-conjugated anti-digoxigenin antibody detection procedures (Boehringer Mannheim).

Fig. 2. Expression of hsc70 and neuron-specific enolase mRNAs in deep cerebellar neurons. **A:** Brightfield microscopy of the deep white matter of the control cerebellum. Darkfield microscopy of adjacent tissue sections hybridized with ^{35}S -labeled hsc70 (**B**) and nse (**C**) riboprobes reveal similar patterns of expression in the deep white matter (DWM). At higher

magnification, hsc70 mRNA and nse mRNA was localized to individual deep cerebellar neurons (DCN). **D** (hsc70) and **F** (nse) focus on cell nuclei; **E** (hsc70) and **G** (nse) focus on the overlying autoradiographic grains. g, glial cells. Bar = 350 μm in A-C, 15 μm in D-G.

RESULTS

Northern Blot Analysis

A characterization by Northern blot analysis of the four riboprobes utilized in the present investigation is shown in Figure 1. The constitutive hsc70 riboprobe detected a 2.5-kilobase (kb) mRNA species in the cerebral hemispheres (CH), cerebellum (CB), and kidney (KID) of both control (C) and 1-hour hyperthermic rabbits (HS). The inducible hsp70 riboprobe detected no mRNA in control brain or kidney, but induction of a 2.7-kb

mRNA species was observed following 1 hour hyperthermic treatment. The hsp70 riboprobe did not cross-hybridize to the constitutive 2.5-kb hsc70 transcript; thus it is highly specific in detecting expression of stress-inducible members of the hsp70 multigene family.

The neuron-specific enolase (nse) riboprobe and the oligodendrocyte-specific proteolipid protein (plp) riboprobe detected mRNA species of 2.3 kb and 3.2 kb, respectively, in brain regions isolated from both control and 1-hour hyperthermic animals. However, signal was not observed in kidney, confirming that these riboprobes

Fig. 3. Hsp70 mRNA and proteolipid protein mRNA in the deep white matter of the cerebellum. Adjacent sections of the cerebellum of 1-hour hyperthermic animals hybridized with ^{35}S -UTP-labeled hsp70 (A) and plp (B) riboprobes. Oligodendrocytes are indicated by the arrows. Bar = 25 μm .

were specific in detecting the transcription of genes whose expression is unique to the nervous system.

Deep White Matter of the Cerebellum

In control cerebellum, constitutive expression of hsc70 mRNA was observed in deep cerebellar neurons. Identification of the different neural cell types relied on two criteria: morphology and expression of nse mRNA or plp mRNA, an oligodendrocyte marker. Figure 2A shows a low-magnification brightfield view of the deep white matter of the cerebellum. Darkfield microscopy revealed similar patterns of expression for hsc70 mRNA (Fig. 2B) and nse mRNA (Fig. 2C) in the deep white matter (DWM) of the cerebellum. At higher magnification (Fig. 2D–G) it was apparent that the hsc70 mRNA and nse mRNA were localized to individual deep cerebellar neurons (DCN). The results for the hsc70 riboprobe are shown in the upper panels and those for the nse riboprobe are in the lower panels. Figure 2D and F focus

on cell nuclei while E and G focus on the overlying autoradiographic grains. Expression of hsc70 mRNA and nse mRNA was not detected in the surrounding glial cell types (g). The data shown in Figure 2 were obtained using in situ hybridization with ^{35}S -labeled riboprobes. As shown by colour photography in Figure 7, improved signal resolution was apparent when non-radioactive DIG-UTP-labeled riboprobes were employed for in situ hybridization. The hsc70 mRNA was highly localized to the cytoplasm of individual deep cerebellar neurons (DCN) (Fig. 7A). Counterstaining with neutral red for identification of nuclei revealed that surrounding glial cells (g) were negative for the hsc70 mRNA while neurons were positive (DCN) (Fig. 7B). No signal was detected in tissue sections hybridized with sense hsc70 riboprobe (Fig. 7C).

Following 1 hour of hyperthermia, a strong induction of hsp70 mRNA was observed in cells in the deep white matter of the cerebellum using the hsp70 ^{35}S -labeled riboprobe (Fig. 3A). This population of cells also showed strong expression of oligodendrocyte-specific plp mRNA (Fig. 3B). At higher magnification (Fig. 7D,E), the non-radioactive DIG procedure permitted localization of the inducible hsp70 mRNA to cytoplasmic cap areas (blue precipitate) at both poles of oligodendrocytes (O). The oligodendrocyte nuclei are visualized with neutral red counterstaining (Fig. 7E).

Molecular Layer of the Cerebellum

Similar patterns of expression of hsc70 mRNA and nse mRNA (Fig. 4A and B, respectively) were noted in adjacent sections of the molecular layer of the cerebellum (ML) suggesting constitutive neuronal expression of hsc70 mRNA in this layer. Constitutive hsc70 mRNA and nse mRNA expression was also observed in the underlying Purkinje neurons (PN). It was difficult to localize signal to individual cells within the molecular layer using radioactive riboprobes. The non-radioactive DIG procedure permitted the localization of hsc70 mRNA to stellate neurons (SN, Fig. 7F) in the outer region of the molecular layer and basket cell neurons (BCN, Fig. 7G) in the inner region of this layer. In the underlying Purkinje layer, hsc70 mRNA was detected in Purkinje neurons (PN), whereas surrounding Bergmann glial cells (g) were hsc70 mRNA negative (Fig. 7G).

Following heat shock, an induction of hsp70 mRNA was observed within oligodendrocytes of the molecular layer (Fig. 5). Focusing on either the cell nuclei (A and C) or on overlying grains (B and D), the upper panels show the localization of the radioactive signal for hsp70 mRNA, whereas the lower panels show the parallel pattern of the oligodendrocyte-specific plp mRNA. The non-radioactive DIG procedure allowed better localization of the inducible hsp70 mRNA to individual oli-

Fig. 4. Distribution of hsc70 mRNA and nse mRNA in the molecular layer of the cerebellum. Low-magnification darkfield microscopy of adjacent tissue sections reveals similar expression patterns for hsc70 (A) and nse (B) mRNAs in the molecular layer (ML). Constitutive hsc70 and nse mRNA expression is also localized to underlying Purkinje neurons (PN). PL, Purkinje layer; GCL, granule cell layer. Bar = 66.7 μ m.

godendrocytes (Fig. 7H), specifically to cytoplasmic cap areas on either side of the nucleus of these cells.

Brainstem

In control and heat shock brainstem, distinctive patterns of expression for constitutive hsc70 mRNA and stress-inducible hsp70 mRNA were observed. In control brainstem, similar patterns of expression for hsc70 mRNA and nse mRNA were observed (Fig. 6A and 6B, respectively) suggesting expression of hsc70 mRNA in neurons (N) while an hsc70 mRNA signal was not detected in surrounding glial cells (g). The non-radioactive DIG procedure confirmed the expression of hsc70 mRNA in neurons and the lack of signal in surrounding glial cells (Fig. 7I,J). These results demonstrate a highly localized pattern of hsc70 mRNA in the cytoplasm of neuronal cell bodies in the brainstem.

In 1-hour hyperthermic animals, a prominent induction of hsp70 mRNA was noted in a cell population which was positive for plp mRNA (Fig. 6C,D). Neighbouring neuronal cells (N) showed no detectable induction of hsp70 mRNA. At higher magnification using the DIG procedure, this induced hsp70 mRNA signal was localized to the cytoplasm of oligodendrocytes (O) (Fig. 7K,L).

DISCUSSION

In this study the expression of constitutive hsc70 mRNA and hyperthermia-inducible hsp70 mRNA has been characterized in cellular layers of the rabbit cerebellum and in the brainstem using radioactive and non-

radioactive in situ hybridization procedures. The non-radioactive in situ hybridization procedure, employing DIG-UTP labeled riboprobes and antibody detection of signal, permits improved signal localization. Using a 35 S-labeled riboprobe, a strong induction of hsp70 mRNA was noted in a cell population in the deep white matter of the cerebellum which demonstrated expression of mRNA encoding proteolipid protein (plp), an oligodendrocyte marker. Cells in the molecular layer of the cerebellum and in the brainstem which express plp mRNA, also demonstrated a prominent induction of hsp70 mRNA in the 1-hour hyperthermic animal.

The non-radioactive DIG in situ hybridization procedure was adopted because it allowed a better localization of stress-inducible hsp70 mRNA to individual oligodendrocytes in the molecular layer and deep white matter of the cerebellum and in the brainstem. The presence of inducible hsp70 mRNA, visualized as a blue precipitate, was apparent in cytoplasmic cap areas at both poles of oligodendrocyte nuclei which were counterstained red. In addition to a greatly improved ability to localize signal to individual cells in the nervous system, the non-radioactive method yielded rapid results compared to the time delay required for signal development with radioactive riboprobes. Our previous studies on the Purkinje cellular layer of the cerebellum have demonstrated induction, in 1-hour hyperthermic rabbits, of hsp70 mRNA in Bergmann glial cells, a specialized type of astrocyte (Manzerra et al., 1993). The present investigation focuses on a second glial cell type, the oligodendrocyte, and indicates that a robust induction occurs in these cells in the cerebellum and brainstem.

Fig. 5. Localization of hsp70 mRNA and plp mRNA in the molecular layer of the 1-hour hyperthermic cerebellum. A (hsp70) and C (plp) focus on the cell nuclei; B (hsp70) and D (plp) focus on the silver grains overlying the oligodendrocytes (O). Bar = 8.3 μ m.

In situ hybridization was also utilized to identify neural cell types which express constitutive members of the hsc/hsp70 multigene family. Using a 35 S-labeled hsc70 riboprobe, it was apparent that neurons in the deep white matter of the cerebellum and in the brainstem expressed constitutive hsc70 mRNA. Use of a 35 S-labeled neuron-specific enolase riboprobe aided in the identification of the neuronal cell types. Improved signal resolution was obtained with a non-radioactive hsc70 DIG-UTP labeled riboprobe. Hsc70 mRNA was observed to be highly localized to the cytoplasm of individual neurons. Counterstaining with neutral red to identify cell nuclei revealed that signal for hsc70 mRNA was not detected in glial cells. In situ hybridization with the non-radioactive DIG riboprobes was particularly useful for characterizing the expression of heat shock mRNA species in small cell types in layers of the cerebellum. Al-

though we were able to localize hsc70 mRNA with radioactive riboprobes to large neurons in the brainstem and cerebellar deep white matter, signal scatter made this difficult for smaller cells such as basket and stellate neurons in the molecular layer of the cerebellum. The non-radioactive DIG procedure permitted detection of constitutive hsc70 mRNA in these neuronal cell populations.

Previous studies have reported that hsc70 protein is abundant in brain tissue, comprising 1% of the soluble protein content (Schlossman et al., 1984). In fact neural tissue has been widely utilized as a rich source for the purification of constitutive hsc70 protein (Ungewickell, 1985; Chappell et al., 1986). Our present and previous studies demonstrate that hsc70 mRNA and protein are localized to neuronal cell populations, whereas signal is not detectable in glial cells (Manzerra and Brown, 1992a,b; Manzerra et al., 1993). As we have suggested,

Fig. 6. Expression of hsc70, hsp70, nse, and plp mRNA in control and heat shock brainstem. Hsc70 mRNA (A) was localized to brainstem neurons (N). The pattern of expression was similar to that observed for nse mRNA (B). Constitutive expression was not detected in surrounding glial cells (g). Following 1 hour of hyperthermia, hsp70 mRNA (C) was induced in glial cells (g) of the brainstem and not in brainstem neurons (N). The pattern of expression was comparable to that observed for plp mRNA (D). Bar = 35.7 μ m.

hsc70 protein likely plays an important role in the normal metabolic functioning of neurons (Brown, 1990, 1994).

The present results indicate that oligodendrocytes located in the brainstem and in the molecular layer and deep white matter of the cerebellum show a robust induction of the hsp70 mRNA in 1-hour hyperthermic animals, whereas the neuronal cell types which we examined in this study do not. These neuronal cell populations, however, show an abundant signal for the constitutive hsc70 mRNA. Endogenous levels of hsc/hsp70 protein may influence the induction of hsp70 during the stress response (Morimoto et al., 1993; Hightower and Li, 1994; Mifflin and Cohen, 1994), perhaps by reducing the extent of activation of heat shock transcription factor (Mosser et al., 1993). We have previously suggested that high levels of constitutive hsc70 in Purkinje neurons may exert a feedback mechanism which initially dampens the induction of hsp70 mRNA following heat

shock (Manzerra et al., 1993). Our present observations on additional neuronal cell types provide further support for a correlation between constitutive hsc70 expression and subsequent induction of hsp70 mRNA after 1 hour of hyperthermia. Neurons which exhibit abundant constitutive expression of hsc70 do not induce hsp70 mRNA at 1 hour; however, we have noted a delayed accumulation of this mRNA in neurons at 10 hours post-heat shock (Manzerra et al., 1993). Conversely, oligodendrocytes, which show no detectable signal for hsc70 mRNA, demonstrate a strong induction of hsp70 mRNA in 1-hour hyperthermic animals.

ACKNOWLEDGMENTS

This research was supported by grants to I.R.B. from the Medical Research Council of Canada. Portions of these results were presented in Foster et al. (1993).

Fig. 7. Cellular localization of hsc70 and hsp70 mRNAs using digoxigenin-UTP-labeled riboprobes. **A:** Control cerebellum showing hsc70 mRNA expression localized to an individual deep cerebellar neuron (DCN). **B:** Adjacent tissue section of control cerebellum hybridized with the hsc70 DIG-UTP riboprobe and counterstained with neutral red. The hsc70 mRNA is localized to the deep cerebellar neuron (DCN) and is not detectable in the surrounding glial cells (g). **C:** No detectable signal was observed on sections hybridized with hsc70 sense riboprobe. DCN, deep cerebellar neuron; g, glial cell. **D:** One-hour hyperthermic cerebellum showing hsp70 mRNA induction in oligodendrocyte (O) in the deep white matter of the cerebellum. **E:** Adjacent section of heat shock cerebellum counterstained with neutral red to show the nuclear morphology of the oligodendrocyte (O) expressing hsp70 mRNA. **F:**

Control cerebellum showing hsc70 mRNA localized to stellate neurons (SN) of the molecular layer. **G:** Control cerebellum showing hsc70 mRNA localized to basket cell neurons (BCN) of the molecular layer, and to Purkinje neurons (PN) of the underlying Purkinje layer, whereas no hsc70 mRNA was detected in Bergmann glial cells (g). **H:** Hsp70 mRNA induction in oligodendrocyte (O) of the molecular layer following 1 hour of hyperthermia. **I:** Low magnification of hsc70 mRNA expression in the control brainstem. **J:** Localization of hsc70 mRNA expression to an individual brainstem neuron. **K:** Hsp70 mRNA expression following 1 hour of hyperthermia in oligodendrocytes (O). **L:** Adjacent tissue section counterstained with neutral red to show nuclear morphology of the oligodendrocyte expressing hsp70 mRNA. Bar = 10 μ m in A–L, 66 μ m in I.

REFERENCES

- Barbe MF, Tytell M, Gower DJ (1988): Hyperthermia protects against light damage in the rat retina. *Science* 241:1817-1820.
- Beckmann RP, Mizzen LA, Welch WJ (1990): Interaction of hsp70 with newly synthesized proteins: implications for protein folding and assembly. *Science* 248:850-854.
- Black MM, Chestnut MH, Pleasure IT, Keen JH (1991): Stable clathrin: uncoating protein (hsc70) complexes in intact neurons and their axonal transport. *J Neurosci* 11:1163-1172.
- Brown IR (1990): Induction of heat shock (stress) genes in the mammalian brain by hyperthermia and other traumatic events: a current perspective. *J Neurosci Res* 27:247-255.
- Brown IR (1994): Induction of heat shock genes in the mammalian brain by hyperthermia and tissue injury. In Mayer RJ, Brown IR (eds): "Heat Shock and the Nervous System." London: Academic Press, pp 31-53.
- Chappell TG, Welch WJ, Schlossman DM, Palter KB, Schlesinger MJ, Rothman JE (1986): Uncoating ATPase is a member of the 70 kilodalton family of stress proteins. *Cell* 45:3-13.
- Chirico WJ, Waters MG, Blobel G (1988): 70K heat shock related proteins stimulate protein translocation into microsomes. *Nature* 332:805-810.
- Cosgrove JW, Brown IR (1983): Heat shock protein in the mammalian brain and other organs following a physiologically relevant increase in body temperature induced by LSD. *Proc Natl Acad Sci USA* 80:569-573.
- Craig EA (1989): Essential roles of 70 kda heat inducible proteins. *Bioessays* 11:48-52.
- Deshaies RJ, Kock BD, Werner-Washburne M, Craig EA, Schekman R (1988): A subfamily of stress proteins facilitates translocation of secretory and mitochondrial precursor polypeptides. *Nature* 332:800-805.
- Forss-Petter S, Danielson P, Sutcliffe JG (1986): Neuron-specific enolase: complete structure of rat mRNA, multiple transcription start sites, and evidence suggesting post-transcriptional control. *J Neurosci Res* 16:141-156.
- Foster JA, Rush SJ, Brown IR (1993): Expression of heat shock genes in the rabbit nervous system. *Soc Neurosci Abstr* 19:1747.
- Georgopoulos C, Welch WJ (1993): Role of the major heat shock proteins as molecular chaperones. *Annu Rev Cell Biol* 9:601-634.
- Gething MJ, Sambrook J (1992): Protein folding in the cell. *Nature* 355:33-45.
- Hightower LE, Li T (1994): Structure and function of the mammalian hsp70 family. In Mayer RJ, Brown IR (eds): "Heat Shock Proteins and the Nervous System." London: Academic Press, pp 1-30.
- Johnston RN, Kucey BL (1988): Competitive inhibition of hsp70 gene expression causes thermosensitivity. *Science* 242:1551-1554.
- Lindquist S, Craig EA (1988): The heat shock proteins. *Annu Rev Genet* 22:631-677.
- Lowenstein DH, Chan PH, Miles MP (1991): The stress protein response in cultured neurons: characterization and evidence for a protective role in excitotoxicity. *Neuron* 7(6):1053-1060.
- Manzerra P, Brown IR (1990): Time course induction of a heat shock gene (hsp70) in the rabbit cerebellum after LSD *in vivo*: involvement of drug-induced hyperthermia. *Neurochem Res* 15(1):53-59.
- Manzerra P, Brown IR (1992a): Distribution of constitutive and hyperthermia-inducible heat shock mRNA species (hsp70) in the Purkinje layer of the rabbit cerebellum. *Neurochem Res* 17:559-564.
- Manzerra P, Brown IR (1992b): Expression of heat shock genes (hsp70) in the rabbit spinal cord: localization of constitutive and hyperthermia-inducible mRNA species. *J Neurosci Res* 31:606-615.
- Manzerra P, Rush SJ, Brown IR (1993): Temporal and spatial distribution of heat shock mRNA and protein (hsp70) in the rabbit cerebellum in response to hyperthermia. *J Neurosci Res* 36:480-490.
- Mifflin LC, Cohen RE (1994): Hsc70 moderates the heat shock (stress) response in *Xenopus laevis* oocytes and binds to denatured protein inducers. *J Biol Chem* 269:15718-15723.
- Milner RJ, Lai C, Nave K-A, Lenoir D, Ogata J, Sutcliffe G (1985): Nucleotide sequences of two mRNAs for rat brain myelin proteolipid protein. *Cell* 42:931-939.
- Morimoto RI (1993): Cells in stress: transcriptional activation of heat shock genes. *Science* 259:1409-1410.
- Morimoto RI, Tissieres A, Georgopoulos C (eds) (1990): "Stress Proteins in Biology and Medicine." Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Mosser DD, Duchaine J, Massie B (1993): The DNA-binding activity of the human heat shock transcription factor is regulated *in vivo* by hsp70. *Mol Cell Biol* 13:5427-5438.
- Nover L (1991): Induced thermotolerance. In Nover L (ed): "Heat Shock Response." Boca Raton, FL: CRC Press, Inc., pp 409-452.
- Pelham HRB (1986): Speculations on the functions of the major heat shock and glucose regulated proteins. *Cell* 46:959-961.
- Pelham HRB (1988): Heat shock proteins: coming in from the cold. *Nature* 332:776-777.
- Riabowol KT, Mizzen LA, Welch WJ (1988): Heat shock is lethal to fibroblasts microinjected with antibodies against hsp70. *Science* 242:433-436.
- Rordorf G, Koroshetz WJ, Bonventre IV (1991): Heat shock protects cultured neurons from glutamate toxicity. *Neuron* 7(6):1043-1051.
- Schlossman D, Schmid S, Braell N, Rothman J (1984): An enzyme that removes clathrin: purification of an uncoating ATPase. *J Cell Biol* 99:723-733.
- Sprang GK, Brown IR (1987): Selective induction of a heat shock gene in the fibre tracts and cerebellar neurons of the rabbit brain detected by *in situ* hybridization. *Mol Brain Res* 3:89-93.
- Ungewickell E (1985): The 70-kDa mammalian heat shock proteins are structurally and functionally related to the uncoating protein that releases clathrin triskelia from coated vesicles. *EMBO J* 4:3385-3391.
- Walsh DA, Klein NW, Hightower LE, Edwards MJ (1987): Heat shock and thermotolerance during early rat embryo development. *Teratology* 36:181-191.
- Walsh DA, Li K, Speirs J, Crowther CE, Edwards MJ (1989): Regulation of the inducible heat-shock 71 genes in early neural development of cultured rat embryos. *Teratology* 40:321-334.
- Walsh DA, Li K, Zeng F, Zhe L, Edwards M (1994): Heat shock genes and cell cycle regulation in early mammalian development. In Mayer RJ, Brown IR (eds): "Heat Shock Proteins in the Nervous System." London: Academic Press, pp 123-144.
- Watowich SS, Morimoto RI (1988): Complex regulation of heat shock and glucose-responsive genes in human cells. *Mol Cell Biol* 8:393-405.
- Welch WJ, Mizzen LA, Arrigo A-P (1989): Structure and function of mammalian stress proteins. In Pardue ML, Feramisco JR, Lindquist S (eds): "Stress Induced Proteins." New York: Alan R. Liss, Inc., pp 187-202.
- Wu B, Hunt C, Morimoto RI (1985): Structure and expression of the human gene encoding major protein hsp70. *Mol Cell Biol* 5:330-341.