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The *N*-ethylmaleimide-sensitive fusion protein (NSF) is preferentially expressed in the nervous system

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Abstract

NSF and SNAPs (soluble NSF attachment proteins), originally identified as cytosolic components of intracellular vesicular transport mechanisms, have recently been implicated in Ca^{2+}-triggered neurotransmitter release from synaptic terminals. Here, we have investigated the temporal and spatial expression pattern of the rodent NSF and SNAP genes. A single transcript of 4.5 kb is highly expressed in rat brain, whereas only minor amounts of NSF mRNA are found in liver, kidney, heart, lung and skeletal muscle. In situ hybridisation revealed NSF transcripts as early as embryonic day 10 preferentially in the nervous system of mouse embryos. In the adult brain NSF is widely expressed with particularly high levels in the hippocampus. An identical expression profile was observed for α/β-SNAP. Our data are consistent with a central function of NSF and SNAPs in neurotransmission.

Key words: Mouse; Development; Hippocampus; NSF; SNAP

1. Introduction

The *N*-ethylmaleimide-sensitive fusion protein (NSF) purified from Chinese hamster ovary cells (CHO cells) is essential for the fusion of transport vesicles with Golgi cisternae in a cell-free assay [1,2]. More recently, NSF has been shown to form a high-molecular weight fusion complex (20 S complex) together with several cytosolic and membrane proteins [3–5]. This complex contains, in addition to NSF, the SNAPs (soluble NSF attachment proteins) and the SNAP receptors [6–8] (SNAREs). Assembly of the 20 S complex is thought to direct vesicles to their target compartment and initiate membrane fusion [6,8,9]. The specificity of this otherwise ubiquitous fusion mechanism was postulated to occur through compartment-specific SNAREs found on the vesicle and target membranes. Recently, three well-characterized presynaptic proteins of the plasma membrane (syntaxin and SNAP-25) and the synaptic vesicle (synaptobrevin) were identified as SNAREs [8]. This finding implicates the 20 S complex in the docking of synaptic vesicles at the active zones of synapses and the subsequent Ca^{2+}-triggered release of neurotransmitter by fusion of the vesicle and the plasma membranes. So far, the evidence for this hypothesis has been entirely biochemical. As a first step to shed light on the physiological function of NSF we analysed the temporal and spatial expression pattern of the NSF gene in rat tissues and mouse embryos and compared it to that of α/β-SNAP.

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Abbreviations: NSF, *N*-ethylmaleimide-sensitive fusion protein; PCR, polymerase chain reacton; SNAP, soluble NSF attachment protein; SNARE, SNAP receptor.

2. Materials and methods

2.1. PCR amplification

cDNA was prepared from 1 μg of adult rat brain poly(A)$^+$ RNA using a cDNA synthesis kit (Boehringer, Mannheim) in a final volume of 20 μl according to the manufacturer's specifications. 1 μl aliquots of the cDNA were used for PCR reactions. Oligonucleotides corresponding to amino acids 291–297 (KVVNGPE; AAGGTGGTCAATGGCCAGA) and 461–453 (AELEGLV; ACCAGACCTTCCAGCTCAGC) of the chinese hamster NSF sequence [2], and amino acids 49–55 (ANMFKMA; GCGAACATGTTCAAGATGGC) and 176–182 (EQYQKAI; ATGGCCTTCT GGTACTGCTC) of bovine β-SNAP [10], were used to generate PCR fragments by 30 cycles of denaturation (1 min, 94°C), annealing (1 min, 55°C) and extension (1 min, 72°C). The fragments were cloned into the *Eco*RV site of Bluescript (Stratagene), and the identity of the clones was verified by dideoxy sequencing.

2.2. RNA preparation and Northern hybridization

Poly(A)$^+$ RNA from various rat tissues was obtained from Clontech. 5 μg RNA were separated on a formaldehyde gel and transferred to Hybond-N membrane (Amersham). NMRI mice (Versuchstierzucht Hannover) were mated overnight, and the day of detection of the vaginal plug was designated embryonic day zero (E0). Total RNA from mouse embryos and adult mouse brain was isolated using guanidiniumthiocyanate (GTC) as described previously [11]. Poly(A)$^+$ RNA was prepared using the polyATract kit (Promega). 2 μg RNA were separated on a formaldehyde gel and transferred to positively charged nylon membranes (Boehringer, Mannheim). Membranes were hybridized overnight with randomly ^{32}P-labelled probes in 50% formamide (Fluka), 5 × SSC, 25 mM sodium phosphate buffer, 1% (w/v) SDS at 55°C and washed with 0.1 × SSC, 0.1% (w/v) SDS at 55°C.

2.3. In situ hybridization

In situ hybridization of paraffin sections using ^{35}S-labelled RNA probes was done as described previously [11].

3. Results and discussion

3.1. Tissue distribution and developmental expression

In order to obtain a rodent NSF probe, we amplified by PCR a 486 bp fragment from rat brain cDNA using

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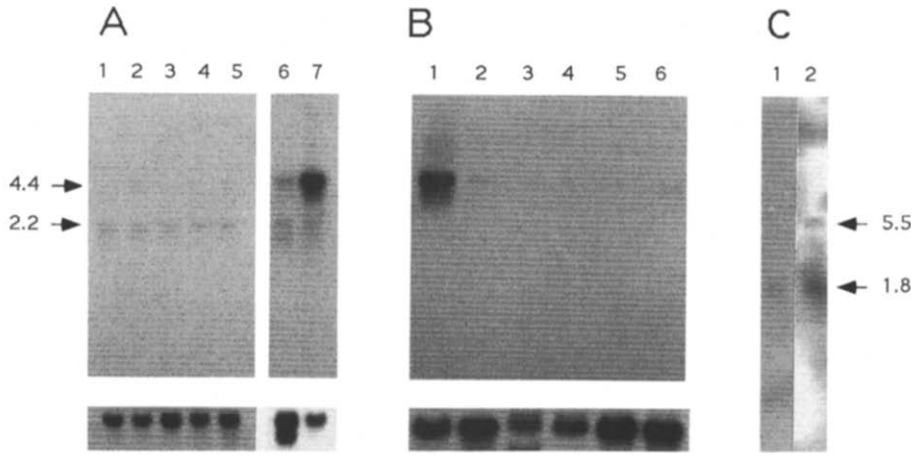

Fig. 1. Expression of NSF and SNAPs in embryos and adult tissues. (A) Poly(A)$^+$ RNA (2 μg per lane) from mouse E10 (lane 1), E11 (lane 2), E12 (lane 3), E13 (lane 4), E15 (lane 5) embryos, newborn (P0) heads (lane 6) and adult brain (lane 7) was hybridized with probes for NSF (upper panel) and γ-actin (lower panel). NSF transcripts of 2.2 kb and 4.5 kb were detected. Hybridization with the γ-actin probe showed that similar amounts of RNA were loaded in all lanes. (B) Poly(A)$^+$ RNA (5 μg per lane) from adult rat brain (1), kidney (2), heart (3), muscle (4), lung (5), and liver (6) were hybridized with probes for NSF (upper panel) and γ-actin (lower panel). (C) Poly(A)$^+$ RNA (1 μg per lane) from mouse E12 (lane 1) and adult brain (lane 2) was hybridized with the β-SNAP probe. Transcripts of 1.8 kb (α-SNAP) and 5.5 kb (β-SNAP) were detected. Sizes are indicated in kb.

primers conserved between Chinese hamster NSF and yeast SEC18 [2]. The conceptual translation of the rat NSF fragment was identical to the amino acid sequence of chinese hamster NSF (data not shown). Similarly, we isolated from rat brain cDNA a 396 bp PCR fragment derived from the β-SNAP mRNA the deduced amino acid sequence (data not shown) of which was identical to that of bovine β-SNAP [10]. Thus, the isolated clones represent the rat NSF and β-SNAP homologs. These probes were used for Northern blot and in situ hybridization experiments.

The size and tissue distribution of the NSF mRNA was determined by Northern blot hybridization. Two transcripts of 2.2 kb and 4.5 kb were detectable in both embryonic and newborn mice. The more abundant smaller transcript is too short to encode a full-length NSF protein (coding sequence of 2,232 bp) and might correspond to an alternatively spliced product of the NSF gene. Alternatively, it could represent a cross-hybidizing mRNA. The size of the high-molecular weight transcript is in good agreement with that of the NSF transcript detected in CHO cells [2]. Low levels of NSF transcripts were visible in mouse embryos as early as embryonic day 10 (E10) and increased gradually until birth (Fig. 1A, lanes 1–5, and see below). A significant difference in expression was seen between newborn and adult brain (Fig. 1A, lanes 6 and 7). Whereas in newborn heads both NSF transcripts were of equal abundance, adult brain showed a significantly higher expression of the 4.5 kb RNA than that of newborn mice.

A single transcript of 4.5 kb was also detected in adult rat brain and, at remarkably constant levels, in all other adult rat tissues examined (Fig. 1B). However, the amount of the NSF mRNA was at least 20-fold lower than in brain. It remains to be analysed if this reflects a low expression in all cells of non-neural tissues, or whether NSF is expressed only in subsets of cells in a given tissue.

NSF has been described as a general factor required for vesicular transport between different cellular compartments [1]. Therefore it should be present in all tissues at varying levels depending on their secretory activity, as has been described for α-SNAP [10]. The preferential expression of NSF in brain, and the low and uniform mRNA levels in other tissues, is therefore surprising. Our data support the idea that NSF has a central function in the regulated release of neurotransmitters. The abundance of NSF transcripts in brain could simply reflect the high demand on vesicle-mediated transport and release that neuronal specialisation implies. However, we favour the idea that the expression profile of NSF indicates its primary function in neurons and that additional genes encoding tissue-specific forms of NSF-related proteins might exist.

The SNAP probe detected a single transcript of 1.8 kb in E12 and E15 mouse embryos (Fig. 1C, lane 1, and data not shown). In newborn mice (P0) and adult brain an additional 5.5 kb transcript was observed (Fig. 1C, lane 2, and data not shown). The probe derived from the β-SNAP RNA contains sequences highly conserved between α- and β-SNAP and should hybridize to both transcripts. The determined transcript sizes are in accordance with those published for bovine α-SNAP (1.8 kb) and β-SNAP (5.6 kb) [10]. β-SNAP is a neuron-specific protein highly homologous to α-SNAP, which functions like α-SNAP in an in vitro assay for intra-Golgi transport

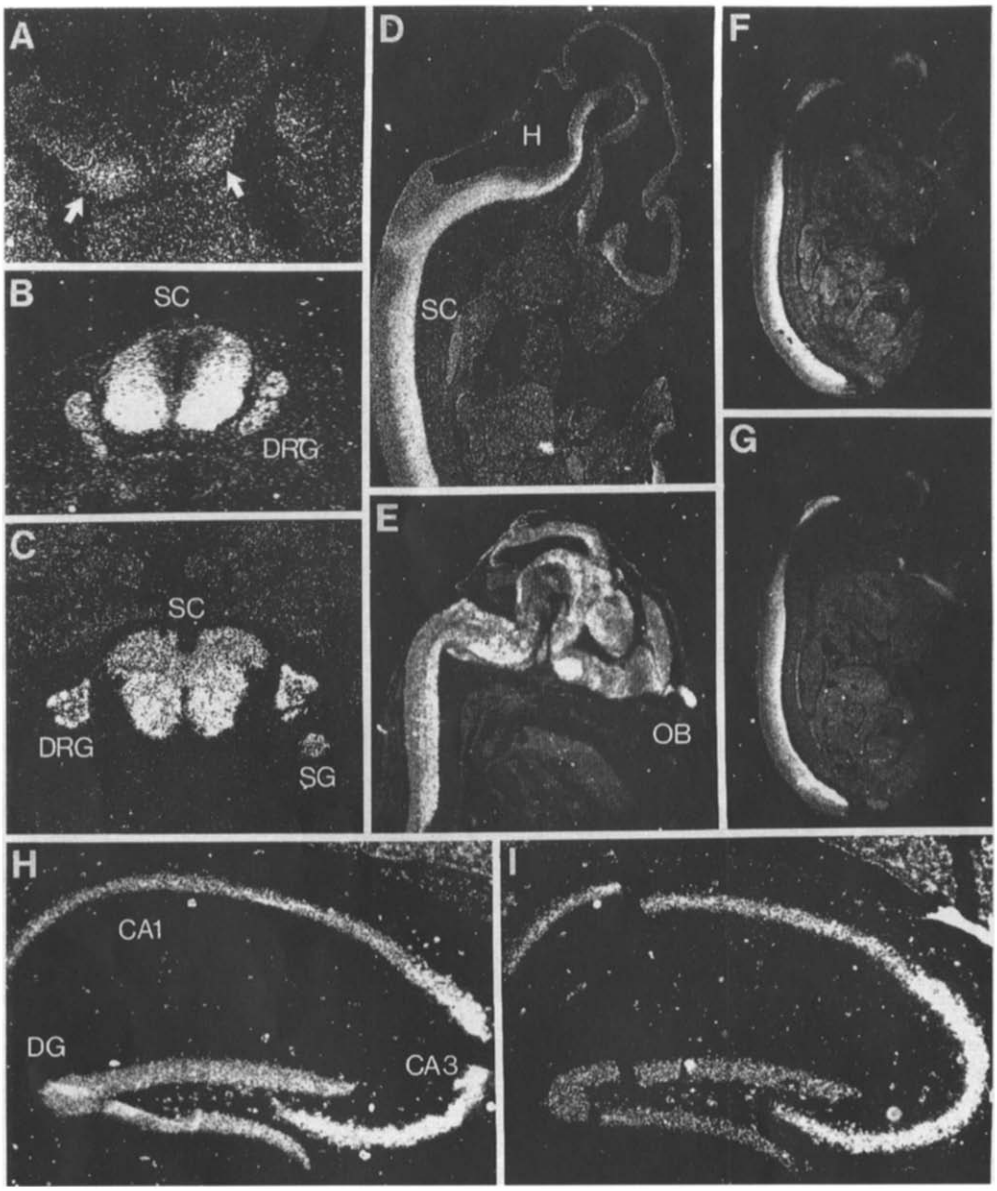

Fig. 2. Distribution of NSF and SNAP transcripts revealed by in situ hybridization. Cross-sections (A–C) and sagittal sections (D–G) of mouse E10 (A), E11 (D), E12 (B,F,G) and E15 (C,E) embryos and sagital sections of adult mouse brain (H,I) were hybridized with NSF (A,F,H) or β-SNAP (B,C,D,E,G) antisense probes. A low level of NSF transcripts is seen as early as E11 (arrows). Expression of NSF and SNAPs is predominantly seen in the nervous system, with high levels of transcripts in the hippocampal formation of the adult brain. Note identical expression patterns of NSF (F,H) and SNAP (G,I) genes in the embryonic CNS (F,G) and the adult hippocampus (H,I). In the embryo only α-SNAP was expressed, whereas in adult brain both α- and β-SNAP were found. The β-SNAP probe detects both transcripts. Dorsal is to the top in A–C,H and to the left in F,G. Rostral is to top in D–G. CA1, CA1 region; CA3, CA3 region; DG, dentate gyrus; DRG, dorsal root ganglion; H, hindbrain; SC, spinal cord; SG, sympathetic ganglion; O, olfactory bulb.

[6,7]. Our results indicate that α-SNAP is detected in mouse embryos as early as E12 whereas β-SNAP seems to be specifically expressed in the adult brain. Thus, β-SNAP may have a specific function in mature neurons.

3.2. *In situ hybridization analysis*

The distribution of NSF and SNAP transcripts in mouse embryos and adult mouse brain was analysed by in situ hybridization with ^{35}S-labeled RNA probes. At embryonic day 10 (E10), low levels of both NSF and SNAP transcripts were detected in the developing nervous system (Fig. 2A, and data not shown). From our Northern analysis we assume that this is due to α-SNAP expression. Both genes, NSF and SNAP, showed an indistinguishable expression pattern throughout development (Fig. 1F,G; see below). Transcripts were predominantly found in the developing nervous system (Fig. 2). At E11 and E12, strong expression of both transcripts was detectable in the brain, spinal cord and dorsal root ganglia (Fig. 2B,D,F,G, and data not shown). In the

spinal cord, signals were mainly seen in the ventral part of the mantle zone where post-mitotic neurons are located. At E15 most of the neurons have been born in the spinal cord [12,13] and both NSF and α-SNAP were expressed at this stage throughout the spinal cord (Fig. 2C,E). In addition a signal was detected in sympathetic ganglia at E15 (Fig. 2C). We did not find prominent expression of NSF outside the nervous system at any stage between E10 and E15. However, it is possible that non-neural cells express transcripts at a low level.

The hybridization pattern in the spinal cord suggests that neurons express NSF and α-SNAP within a day of becoming post-mitotic and at least 1 day before synapses are detectable [14,15]. A SNARE located in the plasma membrane of synapses, SNAP-25, has been found to promote axonal growth, presumably by insertion of membrane via vesicle fusion at the growth cone [16]. The expression of NSF in embryonic post-mitotic neurons prior to synaptogenesis is consistent with a possible function of NSF and α-SNAP in axogenesis similar to that of SNAP-25.

In the adult mouse, NSF expression was seen in most regions of the brain (data not shown). NSF RNA was particularly prominent in the hippocampus (Fig. 2H). A very similar distribution was seen when using a probe for α/β-SNAP (Fig. 2I, and data not shown; [17]). Both the modulation of the efficacy of neurotransmission at existing synapses and the generation of new synaptic connections are thought to contribute to the plasticity of the mature nervous system [18]. Thus, proteins that mediate the docking and fusion of synaptic vesicles at the plasma membrane and/or the insertion of membrane during synaptogenesis are likely targets for regulatory mechanisms. The abundant expression of NSF and SNAP in the hippocampus, a well-characterised model for mechanisms of memory and learning, is therefore highly suggestive of a function of these proteins in neuronal plasticity and allows a direct experimental analysis of this hypothesis.

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