

Research report

Increased expression of synapsin I mRNA in defined areas of the rat central nervous system following chronic morphine treatment

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Abstract

Chronic opiate administration leads to a selective regulation of several cellular proteins and mRNAs. This phenomenon has been viewed as a compensatory mechanism to the opiate signaling leading to the development of opiate addiction. In this study, *in situ* hybridization histochemistry experiments were employed to investigate the effect of chronic morphine treatment on synapsin I gene expression. We show here for the first time that prolonged morphine exposure causes a selective increase in the mRNA levels of synapsin I in several brain regions which are considered to be important for opiate action. Quantitative analysis of the signals, obtained by hybridization of digoxigenin-labeled antisense RNA probe, revealed a 5.8- and 7-fold increase of synapsin I mRNA levels in the locus coeruleus and the amygdala of morphine-treated rats, respectively, as compared with control untreated rats. Increased expression of synapsin I mRNA was also observed in the spinal cord of morphine-treated rats (by 3.8-fold). Since opiates were shown to attenuate neurotransmitter release and reduce synapsin I phosphorylation, it is suggested that the increase in synapsin I levels would lead to the requirement of higher amounts of opiate agonists to obtain the opiate physiological effects. These results suggest that the increases in mRNA levels of synapsin I in these specific areas can be part of the molecular mechanism(s) underlying opiate tolerance and withdrawal.

Keywords: Morphine; Opiate tolerance; Synapsin I mRNA; Locus coeruleus; Amygdala; Spinal cord; *In situ* hybridization

1. Introduction

Opiates exert their biological effects by interacting with three major classes of receptors (δ , μ and κ) [35]. The molecular mechanisms underlying the opiate signal transduction cascade have not been fully established yet. Agonist activation of opiate receptors leads to an attenuation of neurotransmitter release from various neuronal cells [23–25,31], as well as to the inhibition of adenylate cyclase activity [5,9,38] and to the reduction in Ca^{2+} influx through voltage-dependent Ca^{2+} channels [4,15]. We have previously shown that these inhibitory couplings involve pertussis toxin-sensitive GTP-binding proteins (G proteins) [3,4].

Protein phosphorylation plays a pivotal role in signal transduction and in neurotransmitter release [28]. Synapsin I is a phosphoprotein which is predominantly located in nerve terminals where it is associated with the cytoplasmic surface of small synaptic vesicles, binds to the cyto-

skeleton and contributes to the regulation of neurotransmitter release [6,14,40]. The latter is achieved by rapid phosphorylation of synapsin I which is regulated by cAMP-dependent protein kinase and by calcium/calmodulin-dependent protein kinases I and II [14,41]. We have previously shown that acute application of opiate receptor agonists reduces synapsin I phosphorylation in cocultures of rat spinal cord (SC) and dorsal root ganglion (DRG) [27]. This attenuation of synapsin I phosphorylation is in agreement with the inhibition of neurotransmitter release following opiate exposure.

Chronic opiate treatment leads to the development of tolerance and dependence. It has been demonstrated that chronic administration of opiates causes an increase in adenylate cyclase activity in NG108-15 neuroblastoma × glioma hybrid cells [38], enhances cAMP-dependent protein kinase activity in locus coeruleus (LC) [30] and increases the phosphorylation of several proteins, including tyrosine hydroxylase [16]. Alterations in levels of several proteins and mRNAs following chronic morphine treatment have been reported. The levels of $G_{i\alpha}$ and $G_{o\alpha}$ in the

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rat amygdala and LC are increased, while reduction of $G_{i\alpha}$ was found in the nucleus accumbens [30]. A decrease of $G_{i\alpha}$ and $G_{o\alpha}$ subunits was observed in opiate-treated cocultures of SC-DRG and in aggregate cultures of rat forebrain cells [3,43]. Matsuoka et al. [21] reported morphine-induced region-specific increase of type VIII adenylyl cyclase mRNA in rat brain (e.g., in the LC and the amygdala). Elevated levels of cholecystokinin (CCK) mRNA were shown by Pu et al. [32] in amygdaloid neurons. We have recently shown that chronic exposure to opiates resulted in increased levels of synapsin I protein in SC-DRG cocultures [26]. In this study, we investigated the effect of chronic morphine administration on the regional expression of synapsin I mRNA. Increases in synapsin I mRNA were observed in various brain structures and in SC of the opiate-treated adult rat. We would like to propose that the changes in synapsin could have a role in opiate dependence.

2. Materials and methods

2.1. Materials

pSPT18 plasmid containing the 694–2445 fragment of synapsin I cDNA was kindly donated by Dr. L.J. deGennaro (University of Massachusetts Medical Center, Worcester, MA). Restriction enzymes were supplied by Promega (Madison, WI). Radioactive nucleotides were purchased from Amersham (Buckinghamshire, UK). In situ hybridization detection reagents and RNA transcription kit were of Boehringer Mannheim, Germany. All other chemicals were from Sigma (St. Louis, MO).

2.2. Animals and tissue preparation

Adult male Wistar rats (150–200 g) were used in this study. Slow-release morphine base pellets (75 mg each) (NIDA, Rockville, MD) were transplanted subcutaneously, two on the first day, and two on the following day. Three days later, animals were decapitated and the SC and the specific brain structures were removed (for Northern blot analysis). Alternatively, animals were perfused transcardially with 250 ml saline flush followed by 250 ml 4% paraformaldehyde in phosphate-buffered saline (PBS) (for in situ hybridization). Brains and SCs were then removed and postfixed in 4% paraformaldehyde for an additional 48 h at room temperature. Paraffin blocks were prepared by standard methods. Coronal sections of 5 μ m were cut and mounted on poly-L-lysine-coated slides which were stored at room temperature in a dust-free box.

2.3. Synthesis of cRNA probes

pSPT18 plasmid containing synapsin I cDNA (positions 694–2445) was cut using the restriction enzyme *Bam*HI

(for the antisense orientation) or *Sty*I (for the sense orientation). cRNA was transcribed in vitro in the presence of digoxigenin-labeled UTP (Boehringer Mannheim) with either T7 RNA polymerase (antisense) or SP6 RNA polymerase (sense). The sense cRNA had a size of 700 bases. The antisense cRNA was hydrolysed by controlled alkaline digestion (according to the Boehringer Mannheim manual) to yield fragments of 400–500 bases. Both probes were used for in situ hybridization experiments.

2.4. Synthesis of DNA probe

For the preparation of the DNA probe, the plasmid was cut using *Eco*RI and the digestion products displayed on a 0.8% low-melting agarose gel. The 1.7-kb fragment of the synapsin I cDNA sequence was excised from the gel, purified with GeneClean (Bio101, La Jolla, CA) and was labeled with α -[32 P]dCTP by means of the random oligonucleotide priming kit using the Klenow reagent (Fermentas, Vilnius, Lithuania). Unincorporated nucleotides were removed by elution on a BioGel P-60 column (BioRad Lab, Inc., Hercules, CA).

2.5. In situ hybridization

Brain and SC sections were deparaffinated, rinsed twice in $2 \times$ SSC ($20 \times$ SSC: 3 M NaCl, 300 mM sodium citrate) and treated with proteinase K (2 μ g/ml) for 30 min at 37°C. Each section was prehybridized with 100 μ l of prehybridization buffer (50% formamide, $4 \times$ SSC, $2 \times$ Denhardt's solution, 500 μ g/ml salmon sperm DNA and 0.1% sodium dodecyl sulfate [SDS]) for 1–3 h at room temperature. For hybridization, 25 ng of synapsin I digoxigenin-labeled antisense RNA probe was applied in 30 μ l of hybridization solution (equivalent to the prehybridization buffer with the addition of 10% dextran sulfate) for each section. Slides were coverslipped with parafilm and incubated at 42°C for 16 h in a humid chamber. Posthybridization washes were as follows: $4 \times$ SSC (two changes, 15 min each) followed by RNase A treatment (50 μ g/ml) for 30 min at 37°C, $2 \times$ SSC plus 0.1% SDS, $2 \times$ SSC (twice), $1 \times$ SSC (twice), for 10 min each. Following a blocking step (using the blocking reagent supplied in the Boehringer Mannheim DIG detection kit) sections were incubated for 3–5 h at room temperature with anti-digoxigenin antibody conjugated to alkaline phosphatase diluted 1:500 in blocking solution. Color was developed for the appropriate period (to ensure maximal linearity of the reaction) using a mixture of 5-bromo-4-chloro-3-indolylphosphate (0.17 mg/ml) and nitroblue tetrazolium salt (0.34 mg/ml). Reaction was stopped by washing with 10 mM Tris-HCl, pH 7.5, containing 1 mM EDTA followed by ascending concentrations of alcohol and xylene and Permount[®] mounting. Morphine-treated and control sections from parallel brain areas were obtained under the same conditions and were hybridized and stained in paral-

lel. The in situ hybridization signals were viewed and photographed by bright-field microscopy. In order to ensure the specificity of the probe, we performed the following controls on adjacent sections: hybridization with synapsin I digoxigenin-labeled sense RNA probe (see example in Fig. 2D); hybridization in the absence of RNA probe; and RNase A treatment of the section prior to hybridization with the antisense probe.

2.6. Northern blot analysis

Total RNA was extracted from SC and desired brain structures by the method of Chomczynski and Sacchi [10]. RNA was electrophoresed on 1 M formaldehyde-agarose gel (1%). The gel was blotted onto Hybond-N membranes (Amersham) in $20 \times$ SSC. The radioactively labeled synapsin I probe (2×10^7 cpm/ml) was hybridized to the blot at 52°C for 18 h in hybridization solution containing: 0.9 M sodium chloride, 50 mM sodium phosphate, pH 6.5, 50% formamide and 100 $\mu\text{g}/\text{ml}$ denatured salmon sperm DNA. Following hybridization, the washed blot was ex-

posed to Kodak XAR5 film for 4 days at -70°C . Normalization of the amount of synapsin mRNA was obtained by simultaneous hybridization of the blot with a radioactive probe for the mRNA of the housekeeping enzyme, glyceraldehyde phosphodehydrogenase (GAPDH).

2.7. Quantification and analysis

Quantitative analysis of the in situ hybridization of synapsin mRNA was performed with the aid of a computerized video-imaging system (Biological Detection System, Pittsburgh, PA) as previously described [18], with several modifications. Sections from three control and three morphine-treated rats were used in each of the experiments. The number of sections analyzed from each region is denoted by n . For each of the regions studied, the area and the optical density (OD) of the synapsin mRNA signals were determined. The OD values of background levels were subtracted from the synapsin signal OD values. The data for synapsin RNA are presented as OD value multiplied by the area occupied by the signal and are

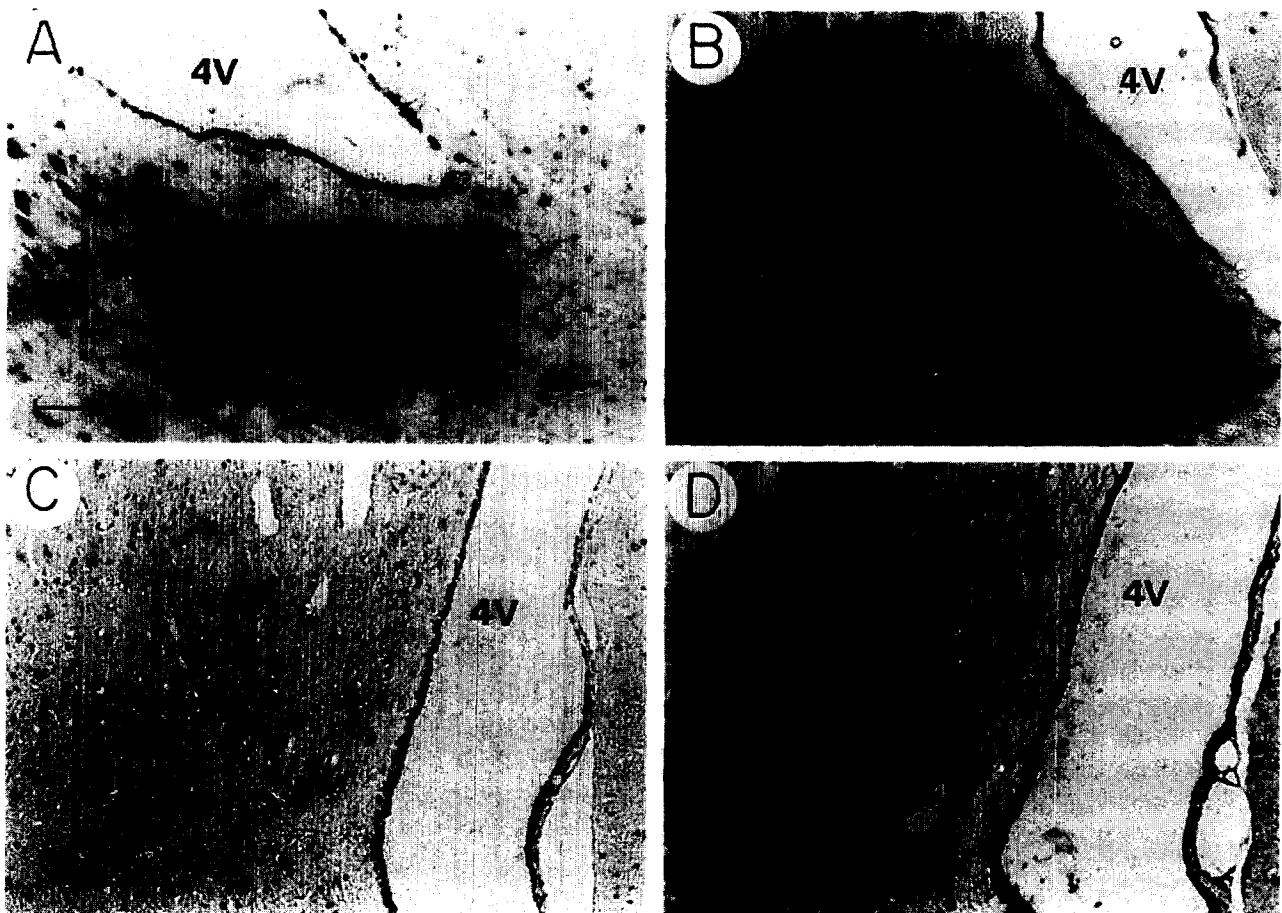


Fig. 1. Comparison of synapsin I mRNA expression in the brain stem of control and morphine-treated rats. Coronal sections of control (A, C) and chronic morphine-treated (B, D) rat brains were hybridized with digoxigenin-labeled antisense cRNA probe for synapsin I (see Materials and methods). A, B: LC; C, D: CGP. Location of the fourth ventricle (4V). Levels of synapsin I mRNA were increased by 5.8 ± 0.1 -fold ($n = 24$ for both control and treated; $P < 0.0001$) in the LC of morphine-treated rats, and by 4.4 ± 0.2 -fold ($n = 14$ for control and $n = 18$ for treated; $P < 0.0001$) in the CGP (as compared with control rats). Bars represent 250 μm .

expressed as fold over control (mean fold \pm SE). Data were analyzed by two-tailed unpaired *t*-test and by Welch's alternate test.

3. Results

The synapsin I gene directs the synthesis of two alternatively spliced mRNAs, encoding synapsin Ia and Ib polypeptides with molecular weights of 74.4 and 70.4 kDa, respectively [40,41]. The cDNA probe used in this study recognizes the common sequence of the two transcripts and labels a 3.4-kb mRNA molecule [22], as was also confirmed by Northern blot analysis of RNA extracted from discrete regions of the rat brain (see Fig. 6 below).

In order to determine the regional distribution of synapsin I mRNA in various brain areas which are known to be relevant to opiate addiction, we performed in situ hybridization experiments using chronic morphine-treated and control rats. Defined matched brain areas were com-

pared for their content of synapsin I mRNA before and after treatment. The changes in the amount of synapsin I mRNA were quantitated using video-computerized imaging.

3.1. The brain stem

In the brain stem, intense signals were present in the LC which is the major noradrenergic nucleus in the brain, located on the floor of the fourth ventricle. Quantitative evaluation of the OD of the in situ hybridization signal revealed major differences between morphine-treated and control brains. A 5.8 ± 0.1 -fold increase in the mRNA levels was observed due to the opiate treatment ($n = 24$ for both control and morphine-treated sections; $P < 0.0001$). High-resolution micrographs (Fig. 1A,B) clearly demonstrate that the signals were markedly increased over most of the cell bodies present in the LC.

Increased expression was also observed in the pontine central gray area (CGP). Chronic morphine treatment pro-

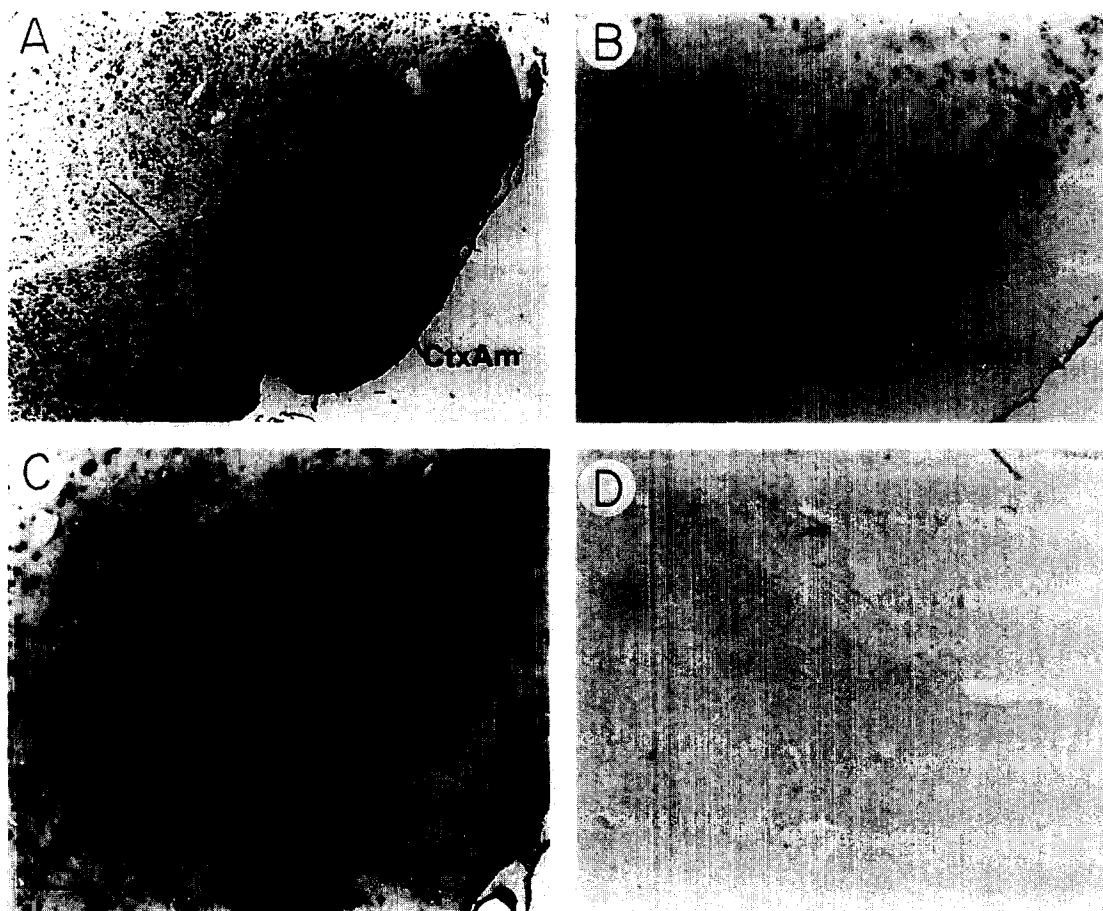


Fig. 2. Synapsin I mRNA in the amygdaloid complex of control and morphine-treated rats. A: An overview of the area of the amygdala of morphine-treated rat hybridized with synapsin I antisense cRNA probe. B: High-resolution micrograph of a representative section from control rat ($n = 11$). C: High-resolution micrograph of a representative section from morphine-treated rat ($n = 15$). D: Micrograph of a neighboring section hybridized with synapsin I sense cRNA probe. Amygdala (Am); cortex-amygdala transition zone (CtxAm). Synapsin I mRNA was increased by 7.0 ± 0.2 - and by 1.7 ± 0.2 -fold ($P < 0.001$) in the amygdala and the cortex-amygdala transition zone of treated rats, respectively (as compared with control rats). Bars represent $250 \mu\text{m}$.

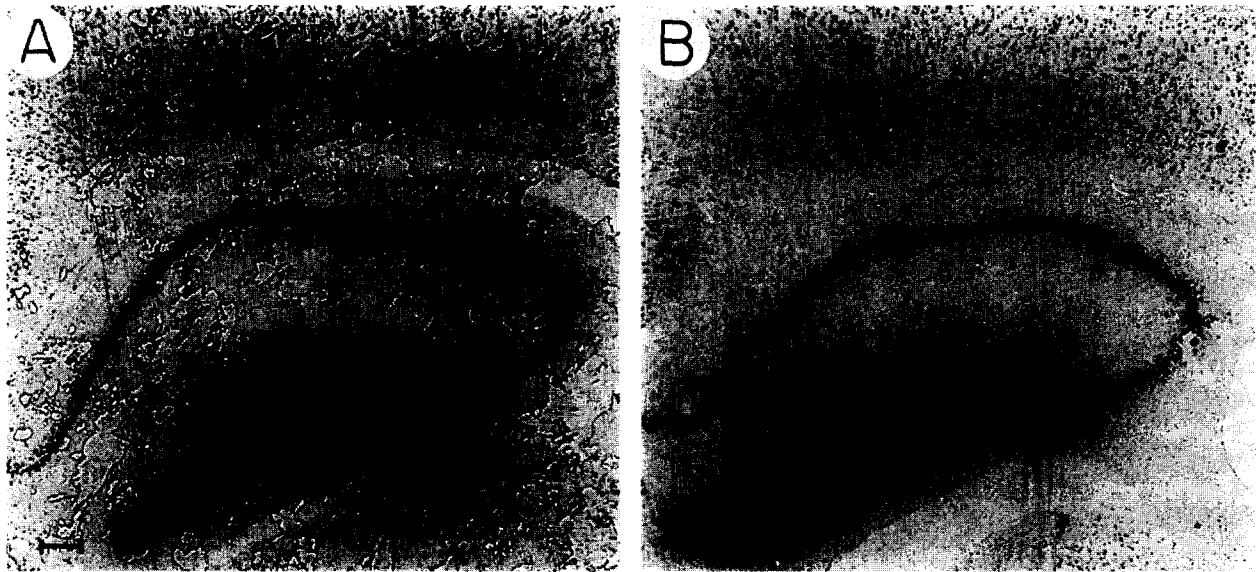


Fig. 3. Synapsin I mRNA in hippocampus of control and morphine-treated rats. Synapsin mRNA in sections from control (A) and chronic morphine-treated rats (B). No significant changes were found in the hippocampus of morphine-treated rats ($n = 7$), as compared with control rats (1.05-fold; $n = 4$). Bars represent 250 μm .

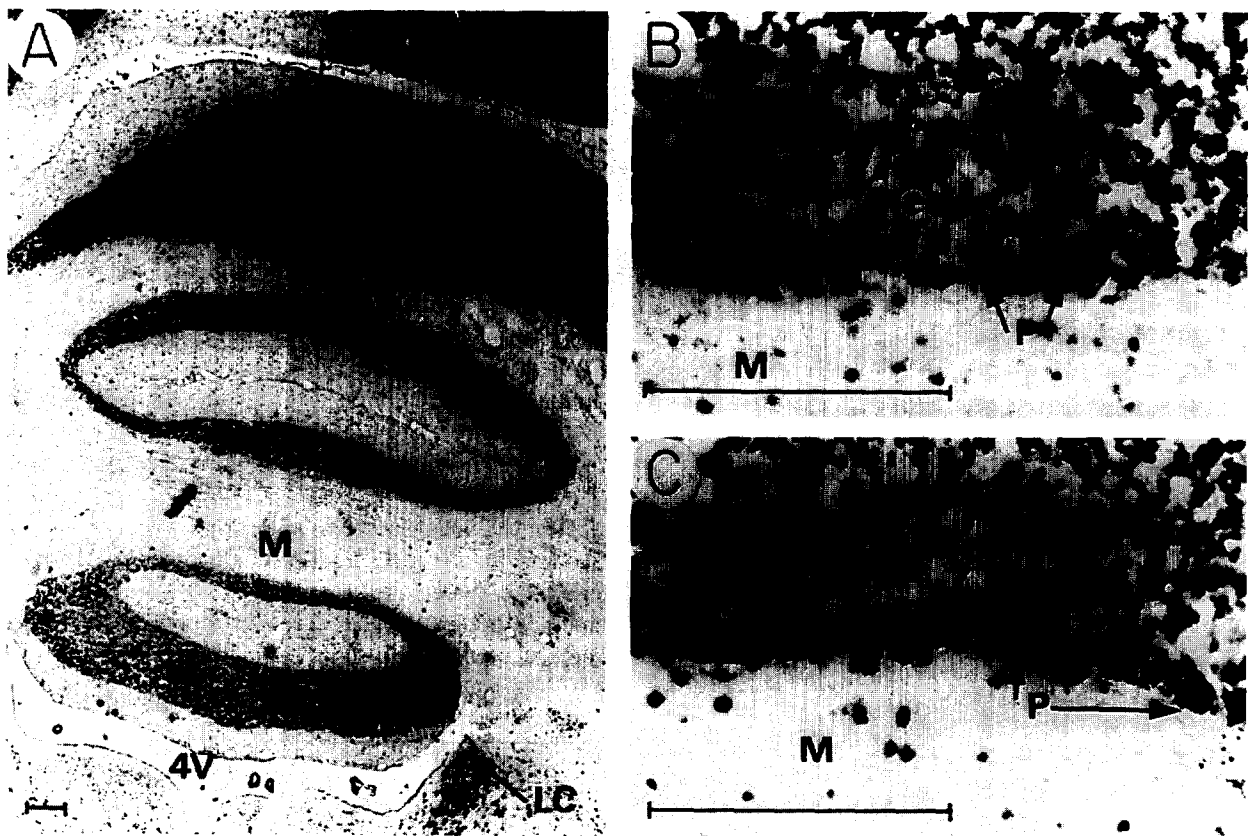
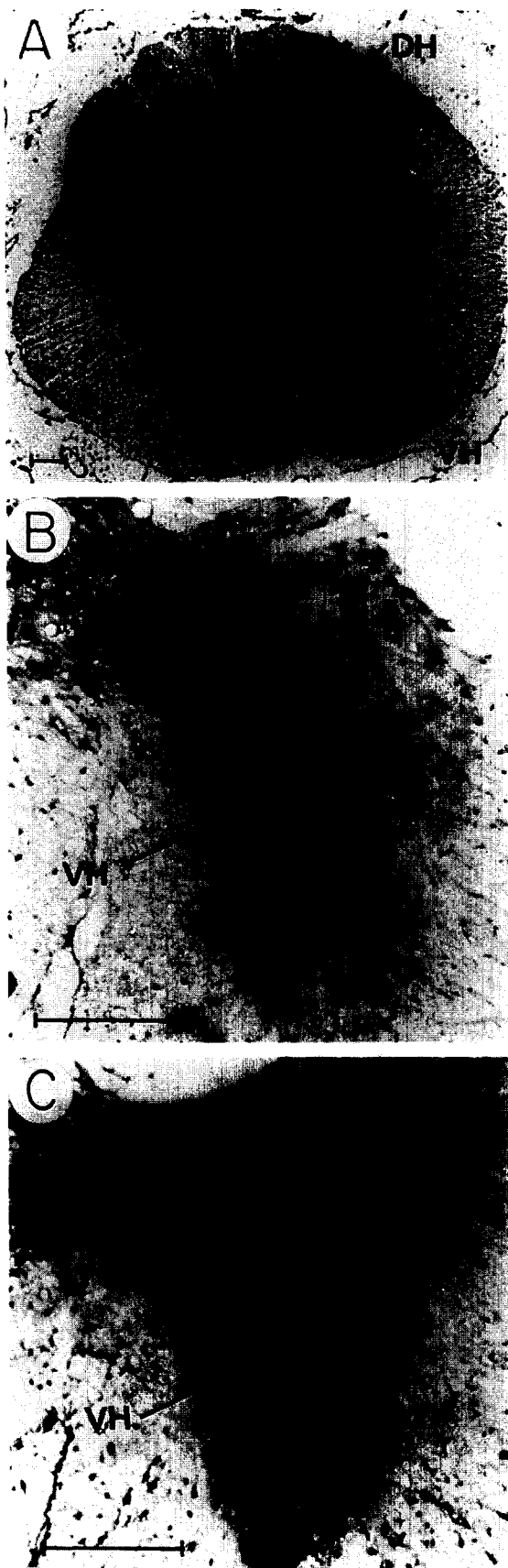


Fig. 4. Synapsin I mRNA in the cerebellum of control and morphine-treated rats. A: An overview of the cerebellum. B: High-resolution micrograph of a control section ($n = 6$). C: High-resolution micrograph of a section from morphine-treated rat ($n = 6$). Granular cell layer (G); Purkinje cells (P); molecular cell layer (M). In situ hybridization signals demonstrate that synapsin I mRNA is distributed predominantly through the granular cell layer and the Purkinje cell layer. No significant changes were observed in the cerebellum of morphine-treated vs. control rats (1.04-fold). Bars represent 250 μm .



duced an increase of 4.4 ± 0.2 -fold in the levels of synapsin I mRNA in this brain structure, as compared with control ($n = 14$ for control and $n = 18$ for treated sections; $P < 0.0001$). Again, most of the increase was evident in cell bodies present in this area (Fig. 1C,D).

Moderate signals of synapsin I mRNA were found in other structures of the brain stem, such as the nucleus of the trapezoid body and the lateral and medial superior olive. However, no significant differences in the mRNA levels of synapsin I were obtained in these areas of morphine-treated brains, as compared with control.

3.2. The telencephalon

Synapsin I mRNA was distributed throughout the amygdaloid complex with the most heavily labeled neurons in the cortex-amygdala (CtxAm) transition zone and in the lateral and basolateral nuclei of the amygdala. Chronic morphine administration caused a 1.7 ± 0.2 -fold increase of the synapsin I mRNA levels in the CtxAm transition zone. An increase of 7.0 ± 0.2 -fold was observed in the nuclei of the amygdala ($n = 11$ for control and $n = 15$ for treated sections; $P < 0.001$) (Fig. 2). Negligible in situ hybridization signal was obtained using the sense cRNA of synapsin (Fig. 2D). This result demonstrates the specificity of the antisense cRNA probe and of the staining method employed.

Of all parts in the brain, the hippocampus showed the highest intensity of synapsin I hybridization signals. However, no significant changes in synapsin I mRNA levels were detected in the hippocampal CA1–4 fields and in the dentate gyrus following morphine treatment [ratio of 1.05 for morphine-treated ($n = 4$) vs. control rats ($n = 7$)] (Fig. 3). Very low hybridization signals were observed in the basal ganglia (caudate putamen) in both control and treated brains. It is worth noting that no significant modifications in synapsin I mRNA levels were observed in these areas even though they are believed to be involved in opiate action.

3.3. The cerebellum

The cerebellar layers were clearly evident following the staining for synapsin I mRNA. The highest labeling was present in the cerebellar granular cell layers and on Purkinje cell bodies. Negligible amounts were detected in the molecular layers. No significant alterations in the levels of

Fig. 5. Synapsin I mRNA in the ventral horn of SC of control and morphine-treated rats. A: An overview of a section from morphine-treated rat SC. B: High-resolution micrograph of the ventral horn from a control rat. C: High-resolution micrograph of the ventral horn from a morphine-treated rat. Dorsal horn (DH); ventral horn (VH). Synapsin I mRNA levels were increased by 3.8 ± 0.1 -fold ($n = 27$ for both control and treated) in the SC of treated as compared with control rats. Bars represent 250 μm .

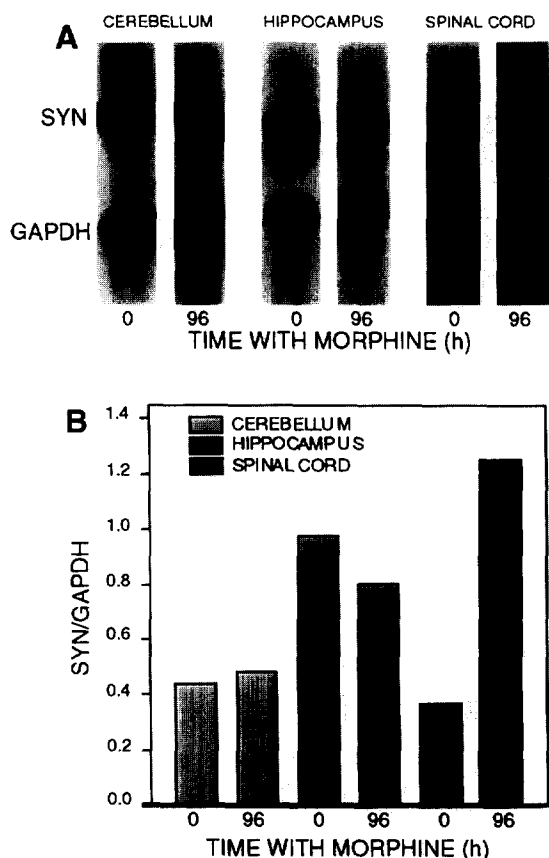


Fig. 6. Northern blot analysis of synapsin I mRNA in defined CNS regions of control and morphine-treated rats. A: Total RNAs from cerebellum, hippocampus and SC were separated by electrophoresis, blotted and hybridized as described in Materials and methods. Left and right panels show synapsin I and GAPDH mRNA hybridizations in regions dissected from control and morphine-treated (96 h) rats, respectively. B: Quantitation of the ratio synapsin/GAPDH mRNA from the representative blot shown in A.

mRNA were found comparing opiate-treated ($n = 6$) and untreated ($n = 6$) cerebellar sections (ratio of 1.04) (Fig. 4). This result is in agreement with the low number of opiate receptors in the rat cerebellum.

3.4. The spinal cord

It is well established that the SC plays a key role in opiate analgesia. This region is rich in opiate receptors and is known to be involved in modulating the effects of noxious stimuli arising from the periphery. We examined synapsin I mRNA levels and pattern of distribution in coronal sections of several subregions of the SC (cervical, dorsal and lumbar). Synapsin I mRNA was predominantly located to the gray matter which includes the cell bodies of motor and sensor neurons. High magnification revealed that the mRNA was found in different cell types originating from both the sensory and the motor systems (the latter are distinguished by their larger size). Chronic morphine treatment resulted in a 3.8 ± 0.1 -fold increase of synapsin

I mRNA levels in cell bodies in the SC ($n = 27$ for both control and morphine-treated sections; $P < 0.0001$). All three subregions described above showed the same ratio (Fig. 5).

3.5. Northern blot analysis

To confirm the data obtained by the in situ hybridization technique and compare the relative levels of expression of synapsin I mRNA in treated and untreated animals, we conducted Northern blot analysis of selected central nervous system (CNS) regions. We performed the experiments using SC, hippocampus and cerebellum from three morphine-treated and three control rats. The mRNA of the housekeeping enzyme GAPDH was hybridized in parallel, in order to normalize the amounts of mRNA applied to the blots. Quantification of the results demonstrated that the levels of synapsin I mRNA (normalized for GAPDH mRNA) were increased by 95% in SC of morphine-treated brains (syn/GAPDH ratio was 0.26 ± 0.09 in three control experiments, vs. 0.92 ± 0.13 in three experiments using treated rats). No significant changes were observed in the cerebellum or hippocampus (Fig. 6).

4. Discussion

This study was directed toward the elucidation of the role of synapsin I in the cascade of complex events leading to the development of opiate tolerance and withdrawal. The data presented above clearly demonstrate that chronic morphine administration causes a specific increase in synapsin I mRNA levels in various regions of the rat CNS, including the LC, CGP, amygdala and SC.

Some aspects of drug addiction can occur relatively rapidly in response to administration of opiates [30]. Nevertheless, most changes in brain function occur gradually in response to prolonged drug exposure. Over the last several years, biochemical and pharmacological studies have shown that chronic opiate treatment leads to the development of tolerance to the acute actions of opiates. Sharma et al. [38] reported opposing effects of acute and chronic opiate exposure on cAMP levels in cultured neuroblastoma \times glioma hybrid cells. These findings and others provided the basis for the notion of cAMP involvement in the development of opiate addiction [9,38]. Indeed, elevated levels of adenylate cyclase activity were reported in rat LC following chronic opiate exposure [12] and it was recently reported that type VIII adenylate cyclase mRNA was increased after such treatment in selective brain regions including the LC and the amygdala [21].

Alterations in opiate signal transduction components have been reported by various groups using various neuronal cells and tissues. Given the role of G proteins in signal transduction, including the cAMP pathway, long-term adaptations in the levels of $G_{i/o}$ and G_s proteins have

been observed in various cell culture models and brain regions, including the amygdala and LC [3,29,30,42–44]. Increased levels of cAMP-dependent protein kinase [30] and of tyrosine hydroxylase [16] were also reported. Chronic morphine also induced increases in CCK mRNA levels in amygdaloid neurons [32]. In this regard, CCK was suggested to act as an endogenous antagonist for morphine analgesia. Taken together, it has been claimed that the regulation of various mRNAs and proteins participating in the opiate receptor's transduction pathway(s) should be regarded as compensatory mechanism(s), leading to the development of opiate dependence [30].

Well defined brain regions are known to mediate the pharmacological and behavioral aspects of opiate drug addiction. The LC plays an important role in opiate physical dependence [1,19] while adaptive changes in the mesolimbic dopaminergic system (ventral tegmental area and the nucleus accumbens) are considered to be the core cause for reinforcement [13]. It was shown that these areas are rich in opiate receptors. LC neurons are regulated by opiate agonists through activation of receptors on both cell bodies and terminal projections [34]. Acutely, opiates inhibit the activity of these neurons via activation of an inward-rectifying K^+ conductance [2] and inhibit the release of noradrenaline [34,46]. Destruction of the LC decreases physical signs of opiate withdrawal [19]. High levels of opiate-binding sites are also distributed through the amygdala [20,33] and through the central gray of the pons [8]. Based on this information, we decided to explore the effect of chronic morphine treatment on synapsin I expression in LC and other selective brain regions which are involved in opiate dependence using the *in situ* hybridization technique.

We have chosen to focus on the levels of expression of synapsin I mRNA since this synaptic vesicle-associated protein is a good candidate for the regulation of neurotransmitter release and its modulation by opiates. In this regard, we have previously demonstrated that acute opiate agonist treatment modulates the phosphorylation state of synapsin I [27] and that chronic exposure to opiate agonists results in increased levels of synapsin I protein [26]. In this study, we found that synapsin I mRNA was differentially distributed throughout the brain, showing the highest levels in the hippocampus. Other areas abundant in synapsin I mRNA were the granular cell layer of the cerebellum, the amygdaloid nuclei, the LC and the CGP. Low levels were detected in the basal ganglia, even though that the basal ganglion is known to contain a variety of interneurons. These findings are compatible with those reported by Melloni et al. [22] and with the recently published work of Zurmohle et al. [47], describing the changes of synapsin I mRNA during rat brain development.

Chronic administration of morphine led to an increase of synapsin I mRNA levels in specific brain structures. A large increase was especially observed in the LC and the amygdala, areas in which increases in the mRNA of type

VIII adenylylase were observed following morphine treatment [21]. It is important to stress that various other regions which could be involved in drug addiction (e.g., hippocampus, caudate nucleus) showed no significant alterations in synapsin I mRNA. Interestingly, according to the work of Matsuoka et al. [21], no changes were observed in the levels of type VIII adenylylase mRNA in the cerebral cortex, nucleus accumbens, caudate putamen and the dorsal raphe nucleus following chronic morphine treatment. This association between adenylylase and synapsin I is intriguing and suggests: (i) that several areas are more prone to opiate regulation than others; (ii) that there may be a common transcriptional regulation for the two proteins; and alternatively (iii) the changes in cAMP in these areas due to increased adenylylase may regulate the expression of cAMP-regulated proteins. Indeed, synapsin I was shown to contain a cAMP responsive element (CRE) in its promoter sequence [37].

The increase in adenylylase in these areas is in agreement with the concept of involvement of phosphorylation in opiate tolerance [28]. In this regard, increased phosphorylation of synapsin I (due to enhanced adenylylase activity and the elevated levels of synapsin protein) could lead to increased neurotransmitter release, as indeed observed under conditions of opiate tolerance and withdrawal [45]. The increase of the OD values of treated brain sections, as compared with control, may reflect an increase in the content of synapsin I mRNA, rather than an elevation in the number of cells that express this mRNA (Fig. 1). This result is in agreement with the report of Greengard et al. [14], which showed that virtually all nerve terminals contain synapsins (see also Fig. 1).

Rosahl et al. [36] have recently shown that knockout of the synapsin I gene in mice did not significantly affect several synaptic functions, although it did lead to alterations in short-term plasticity. In contrast, dramatic changes in development, neurotransmitter release, physiology, morphology and behavior were observed in synapsin I-knockout mice, as compared with control (P. Greengard, personal communication). Moreover, studies by Browning et al. [7] and Lippa and Smith [17] further strengthen the notion that alterations in synapsin I gene expression could accompany the development of abnormalities. They reported reduced levels of synapsin I protein in the hippocampus of schizophrenics [7], as well as in the brains of Alzheimer's disease patients [17]. Melloni et al. [22] reported correlations between the patterns of expression of synapsin I and of another synaptic vesicle protein, synaptophysin. At this stage, we cannot exclude the possibility that other synaptic vesicle-associated proteins (synapsin II, synaptophysin, synaptotagmin, synaptobrevin, etc.) are also involved in opiate tolerance. Thus, further experiments should study the adaptations in these proteins and mRNAs following opiate exposure.

Several cellular adaptations were shown to accompany development of tolerance and dependence following free-

quent utilization of opiates. Taking together the reversal of the inhibition of adenylate cyclase and Ca^{2+} channels [4,5], the increase in mRNA levels of adenylate cyclase [21], the sustained activation of G_s -coupled excitatory opioid receptors [11,39], the increase in activity of cAMP-dependent protein kinase [30], and the increase in synapsin I protein level [26], we could assume that these cellular changes should lead to enhancement of synapsin I phosphorylation. Hence, increased doses of opiate agonists would be needed to inhibit synapsin phosphorylation and attenuate neurotransmitter release (consistent with opiate tolerance). Upon removal of the opiate agonist, the elevated amounts of these cellular components are expected to produce a rapid phosphorylation of synapsin leading to enhanced transmitter release, a process that is known to occur during opiate withdrawal [45].

In conclusion, this study provides evidence that prolonged opiate treatment evokes an increase in synapsin I mRNA, in a region-specific manner. The CNS structures which were significantly influenced (LC, amygdala, CGP, and the SC) are known to be involved in opiate analgesia, tolerance, dependence and withdrawal. Our results suggest that alterations in synapsin I mRNA expression are part of the cellular adaptations that occur during opiate dependence.

Acknowledgements

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