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Decreased levels of preprotachykinin-A and tachykinin NK_1 receptor mRNA in specific regions of the rat striatum after electroconvulsive stimuli

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Abstract

The effects of electroconvulsive stimuli on the expression of mRNAs coding for preprotachykinin-A and the substance P-sensitive tachykinin NK_1 receptor were examined in subregions of the rat striatum. In the electroconvulsive stimuli-treated animals, a 43% decrease in preprotachykinin-A mRNA was detected in the dorso-lateral caudate-putamen as compared to sham electroconvulsive stimuli treated animals. A 75% decrease in numerical density of tachykinin NK_1 receptor mRNA positive neurons was found in the caudal part of the nucleus accumbens core. These findings provide new evidence for selective effects of electroconvulsive stimuli on specific populations of neurons in the rat striatum.

Keywords: Brain; Electroconvulsive stimuli; Hybridization, in situ; mRNA expression; Substance P; Tachykinin receptor; (Rat)

1. Introduction

Substance P is a undecapeptide that belongs to the tachykinin family of peptides, characterized by the carboxy-terminal sequence Phe-X-Gly-Leu-Met-NH_2. Other members are neurokinin A and neurokinin B. Substance P and neurokinin A are both translated from a common mRNA transcribed from a single preprotachykinin-A gene (Krause et al., 1987; Nawa et al., 1984). The tachykinins act on different receptors and the receptor that mediates the action of substance P is the tachykinin NK_1 receptor (Maggi, 1995). Although both substance P and neurokinin A are found in the brain, the receptor expressed in neurons of the central nervous system is mainly of the substance P-sensitive tachykinin NK_1 type (Maggi, 1995). Preprotachykinin-A mRNA expression is affected by psychotropic drugs and specific neuronal lesions. Thus, both preprotachykinin-A mRNAs and substance P are decreased in the striatum after treatment with dopamine D_2 receptor antagonist such as haloperidol (Angulo et al., 1990; Bannon et al., 1986; Lindefors, 1992) or after denervation of the dopaminergic neurons in the striatum (Lindefors et al., 1990, 1989; Young et al., 1986). In addition to being a widely used treatment for severe depressive disorders, electroconvulsive therapy is also efficient in the treatment of psychiatric syndromes assumed to be associated with altered dopamine neurotransmission, i.e. depression with psychotic features and catatonia (Fibiger, 1990). In line with this, improvement in motor function has been observed in patients with Parkinson's disease after electroconvulsive therapy (Anderson et al., 1987); however, the beneficial mechanisms of electroconvulsive therapy action are not well understood. Electroconvulsive stimuli are widely applied in animal studies as a model for electroconvulsive therapy (Green and Nutt, 1987). In this study, we have used this model to examine the mRNA expression of preprotachykinin-A and tachykinin NK_1 receptor in the dorsal and ventral caudate-putamen and in the nucleus accumbens core and shell of the rat brain after electroconvulsive stimuli. We provide evidence for effects of electroconvulsive stimuli on preprotachykinin-A and tachykinin NK_1 receptor mRNA expression in specific brain regions

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assumed to be important for the clinical effects of electroconvulsive therapy.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (ALAB, Sollentuna, Sweden) weighing 200–230 g were used. They were housed at a constant room temperature of 21 ± 1°C with ad libitum access to chow and water and with a 12 h light-dark cycle. Animals were given six daily sham electroconvulsive stimuli ($n = 5$) or electroconvulsive stimuli ($n = 4$) treatments as previously described (Zachrisson et al., 1995) and killed 3 h after the last session. The experiments were approved by the Regional Animal Protection Committee and the rats were kept in accordance with the Karolinska Institute's guidelines.

2.2. In situ hybridization

A 48-mer oligonucleotide encoding amino acids 49 to 64 of the rat tachykinin precursor protein (Krause et al., 1987), thus complementary to α-, β- and γ-preprotachykinin-A mRNA, was used as a probe. A 48-mer oligonucleotide, complementary to the nucleotides in rat tachykinin NK_1 receptor mRNA encoding for amino acids 47 to 95 of the rat tachykinin NK_1 receptor (Yokota et al., 1989), was used to detect tachykinin NK_1 receptor mRNA. The oligonucleotide DNAs were synthesized by Scandinavian Gene Synthesis (Köping, Sweden) and used as hybridization probes. The oligonucleotide probes were labeled and the in situ hybridization experiments with appropriate controls were carried out as described in an earlier study (Zachrisson et al., 1995). The sections hybridized with tachykinin NK_1 receptor oligoprobes were dipped in photo-emulsion, exposed for 3 weeks, developed and counter stained. The sections hybridized with preprotachykinin-A oligoprobes were put on autoradiographic film and exposed for 3 weeks.

2.3. Measurements

The quantification of preprotachykinin-A mRNA on autoradiographic films was chosen since more than 50% of the neurons in the striatum are labeled. However, for tachykinin NK_1 receptor mRNA, the labeling is restricted to a few scattered yet significantly labeled neurons which makes, in this case, analysis of autoradiographic films futile. Therefore, the numerical density of tachykinin NK_1 receptor mRNA hybridization-positive neurons in electroconvulsive stimuli-treated and control animals was determined by using photo-emulsion dipped slides. For the preprotachykinin-A measurements of the autoradiographic films, a light table was equipped with a MTI CCD 72 video camera and the optical density was calculated by means of an Apple Macintosh-based image analysis system (IMAGE v. 1.55, Wayne Rasband, NIMH, Bethesda, MD, USA). Calibration standard with different quantities of radioactivity was included with the tissue sections during exposure of the autoradiographic film. The procedure used provides values corresponding to relative amounts of preprotachykinin-A mRNA (Brené et al., 1993). For the tachykinin NK_1 receptor measurements, the positive neurons were defined as those expressing more than 20 grains per neuron. The sections were analyzed using a Nikon microscope at 280 times magnification. Quantitative analysis of preprotachykinin-A and tachykinin NK_1 receptor mRNA levels in the dorsal caudate-putamen was performed using sections at the level bregma −3.60 mm; the ventral caudate-putamen and the nucleus accumbens were measured at bregma −7.10 mm according to a brain atlas (Paxinos and Watson, 1986) (Fig. 1). For each of the preprotachykinin-A and tachykinin NK_1 receptor mRNA measurements, two adjacent sections per level were quantified, i.e. four sections per animal.

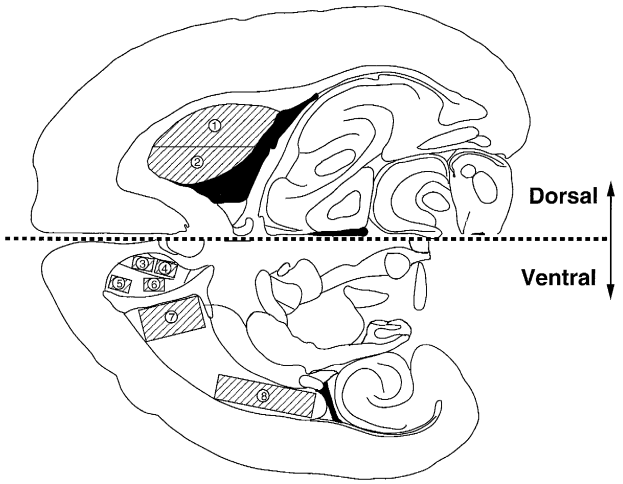

Fig. 1. Schematic drawings of representative sections for measuring preprotachykinin-A (optical density) and tachykinin NK_1 receptor (number of neurons/mm^2) mRNA in the rat striatum. The dorsal section corresponds to bregma −3.60 mm and the ventral section to bregma −7.10 mm, according to a brain atlas (Paxinos and Watson, 1986). 1, dorso-lateral caudate-putamen; 2, dorso-medial caudate-putamen; 3, nucleus accumbens shell rostral; 4, nucleus accumbens shell caudal; 5, nucleus accumbens core rostral; 6, nucleus accumbens core, caudal; 7, ventro-medial caudate-putamen; 8, ventro-lateral caudate-putamen.

2.4. Statistical analysis

The in situ hybridization data are presented either as the mean value (preprotachykinin-A mRNA measurements) or the median (tachykinin NK_1 receptor mRNA) of values for each individual animal. For the measurements of preprotachykinin-A mRNA on autoradiographic films, Student's *t*-test was used for testing significance at the level of $P < 0.05$. For the measurements of numerical density of

tachykinin NK_1 receptor mRNA containing neurons, Mann-Whitney's non-parametric two-group analysis was used for testing significance because of an abnormal distribution of values.

3. Results

3.1. Effects on preprotachykinin-A mRNA expression

Electroconvulsive stimuli decreased the amount of preprotachykinin-A mRNA by 43% ($P < 0.05$) in the dorso-lateral caudate-putamen as visualized on autoradiographic film (Fig. 2). No effects of electroconvulsive stimuli on preprotachykinin-A mRNA levels were seen in any other region examined in the caudate-putamen or in the nucleus accumbens.

3.2. Effects on tachykinin NK_1 receptor mRNA expression

Electroconvulsive stimuli appeared to decrease the numerical density of tachykinin NK_1 receptor mRNA-containing neurons in the dorsal caudate-putamen; however, no statistical difference was found between the groups. In the ventral caudate-putamen no effect on the number of tachykinin NK_1 receptor mRNA positive neurons was found. In the nucleus accumbens core, a 74% decrease in the number of tachykinin NK_1 receptor mRNA positive neurons was found but only in the caudal part ($P < 0.05$). A tendency to a decrease was seen throughout the rostral parts of the nucleus accumbens core as well as in the accumbens shell.

4. Discussion

In this study, in situ hybridization histochemistry was used to examine the effect of electroconvulsive stimuli on preprotachykinin-A and tachykinin NK_1 receptor mRNA levels in the nucleus accumbens and caudate-putamen. We observed decreased levels of preprotachykinin-A mRNA in the dorso-lateral caudate-putamen (Table 1). Earlier studies on substance P and neurokinin A tissue levels after electroconvulsive stimuli have failed to show any change in the striatum (Stenfors et al., 1994, 1992). However, these investigations were made with a lower anatomical resolution and the changes in preprotachykinin-A mRNA levels seen in this study in the dorso-lateral aspect of the striatum might be too localized to significantly change the amount of peptide in the total striatum. Decreased levels of preprotachykinin-A mRNA in the dorso-lateral caudate-putamen may be an indication of decreased dopaminergic tonus,

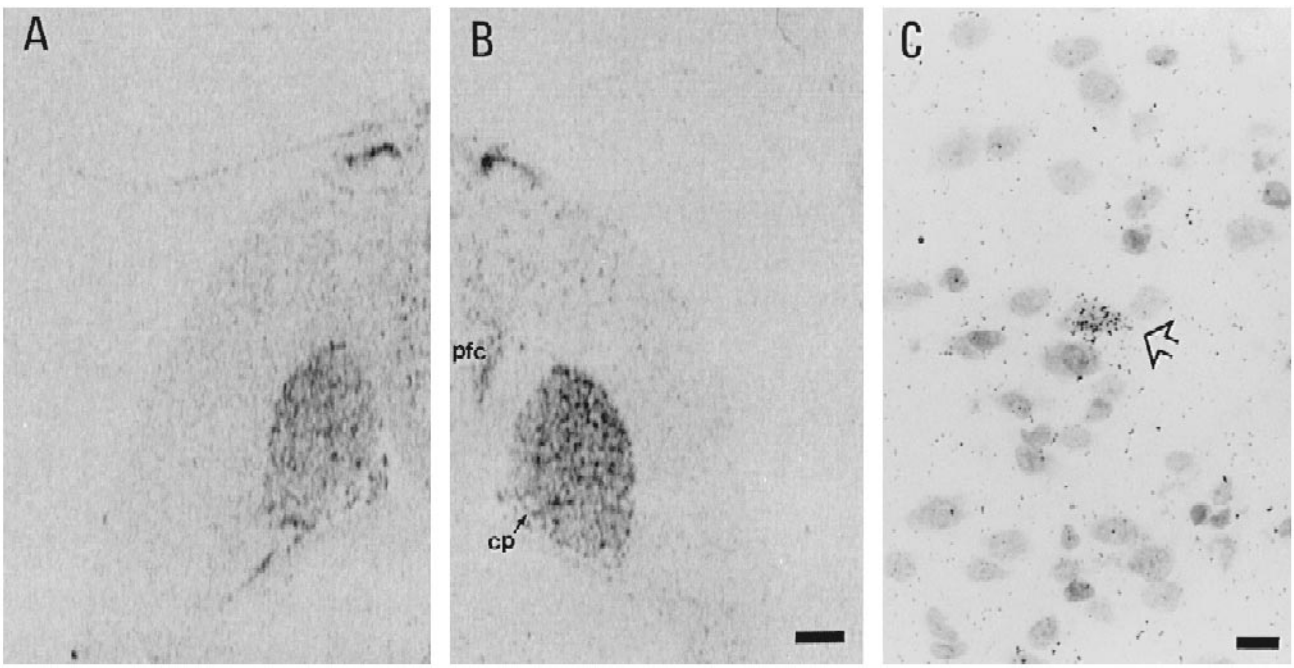

Fig. 2. Autoradiographic film showing the frontal part of a representative horizontal section of rat brain hybridized to a probe detecting preprotachykinin-A mRNA after (A) six electroconvulsive stimuli and (B) six sham electroconvulsive stimuli. Note the significant decrease in labeling intensity in the dorso-lateral caudate-putamen after electroconvulsive stimuli treated as compared to sham treated animals. Scale bar = 1 mm. Abbreviations: cp, caudate-putamen; pfc, prefrontal cortex. (C) A photomicrograph showing a tachykinin NK_1 receptor mRNA containing neuron in the caudal nucleus accumbens as indicated by an open arrow (cell taken from a sham-treated animal). Due to the low numerical density of positive neurons, a comparison between sham electroconvulsive stimuli treated and electroconvulsive stimuli treated animals is difficult to illustrate at this magnification. Scale bar = 10 μm.

Table 1
Optical density measured on autoradiographic films exposed to preprotachykinin-A mRNA hybridized sections and numerical density of tachykinin NK_1 receptor mRNA-expressing neurons in selective regions of the rat brain following six sham electroconvulsive stimuli ($n = 5$) and six electroconvulsive stimuli ($n = 4$) treatments, respectively. In the case of tachykinin NK_1 receptor mRNA-containing neurons, non-parametric statistics were used because of abnormally distributed values

	Preprotachykinin-A mRNA (optical density units)		Tachykinin NK_1 receptor mRNA (positive neurons/mm^2)			
	Sham electroconvulsive timuli (mean ± S.E.)	Electroconvulsive stimuli (mean ± S.E.)	Sham electroconvulsive stimuli		Electroconvulsive stimuli	
			median	min; max	median	min; max
Caudate putamen						
Ventro-medial	31 ± 3.2	29 ± 4.0	1.6	(0.1; 5.9)	1.6	(0.4; 2.3)
Ventro-lateral	56 ± 6.2	48 ± 6.1	2.3	(1.6; 9.4)	1.8	(0.8; 6.5)
Dorso-medial	38 ± 3.1	34 ± 3.9	9.4	(2.4; 12.5)	4.9	(1.6; 9.0)
Dorso-lateral	51 ± 5.4	29 ± 3.9 [a]	7.8	(5.5; 9.0)	2.7	(1.2; 7.8)
Nucleus accumbens						
Core, caudal	26 ± 2.4	22 ± 2.9	3.1	(1.0; 6.2)	0.8 [b]	(0.0; 2.1)
Core, rostral	65 ± 6.1	66 ± 12	4.7	(1.0; 7.3)	1.6	(1.0; 5.2)
Shell, caudal	88 ± 12	85 ± 12	6.3	(1.0; 9.4)	3.7	(0.0; 6.3)
Shell, rostral	74 ± 13	69 ± 13	5.2	(2.1; 6.3)	3.7	(1.6; 7.3)

[a] Significant difference from sham electroconvulsive stimuli-treated animals ($P < 0.05$; Student's *t*-test). [b] Significant difference from sham electroconvulsive stimuli-treated animals ($P < 0.05$; Mann-Whitney non-parametric comparison).

since both dopamine lesions (Brené et al., 1993; Young et al., 1986) and dopamine receptor antagonist administration (Bannon et al., 1986; Lindefors et al., 1992) decrease preprotachykinin-A mRNA in this part of the caudate-putamen. Earlier studies of dopamine receptor sensitivity to dopamine receptor agonist treatment did not clearly indicate a decreased dopaminergic transmission after electroconvulsive stimuli (Green et al., 1977; Modigh, 1975). In these studies, increased dopamine receptor agonist sensitivity was measured. This may be a result of increased receptor sensitization due to decreased endogenous dopaminergic activity or, alternatively, sensitization of receptors by other mechanisms. In addition, electroconvulsive stimuli-induced increase in in vivo release of dopamine in the dorso-lateral caudate-putamen is significantly attenuated following pretreatment with electroconvulsive stimuli (Zis et al., 1991). Thus it appears as if electroconvulsive stimuli in some way decrease stimulation-induced dopaminergic transmission. In line with this, enkephalin and its mRNA are increased by electroconvulsive stimuli (Hong et al., 1979). It is known from dopamine-lesion studies that removal of dopaminergic tonus disinhibits enkephalinergic neurons. This results in increased preproenkephalin levels (Young et al., 1986), by a dopamine D_2 receptor dependent mechanism (Gerfen, 1992). Altogether, evidence indicates that our finding of an electroconvulsive stimuli-induced decrease in preprotachykinin-A mRNA levels in the dorso-lateral caudate-putamen is accompanied by a decreased dopaminergic tonus in this part of the brain.

The striatum is in this context of interest because of its presumed role in the expression of psychomotor activity, reward and motivational behavior, phenomena of importance in affective disorders, catatonia, delirium and parkinsonism. These phenomena may be attenuated or alleviated by electroconvulsive therapy. Electroconvulsive stimuli result in sensitization of dopaminergic mechanisms in the ventral striatum, as shown for both apomorphine- and amphetamine-induced locomotion (Green et al., 1977; Modigh, 1975). Recent observations indicate that the expression of mRNAs for substance P and the dopamine D_1 receptor is influenced by cortical neuronal afferents in a dopamine-dependent fashion (Brené et al., 1993, 1994). This effect was seen in the ventral and medial striatum but not in the dorso-lateral striatum. It has also been shown that dopamine receptor mRNA expression in the ventral striatum is increased by electroconvulsive stimuli (Smith et al., 1995). Interestingly, dopaminergic mechanisms in the ventral striatum are closely connected to locomotion (Sharp et al., 1987), and substance P has a tonic facilitatory influence on dopamine release in this brain region (Elliott et al., 1986). Our observation of a decrease in the numerical density of tachykinin NK_1 receptor mRNA is accordingly an indication of changes in substance P-ergic transmission. The significance of the observed change in tachykinin NK_1 receptor mRNA expression needs to be determined.

The present study provides new evidence for subregion-specific involvement of preprotachykinin-A and tachykinin NK_1 receptor mRNA expression in the striatum in the mechanism of electroconvulsive stimuli and possibly of electroconvulsive therapy. Decreased levels of preprotachykinin-A mRNA may indicate a down-regulated dopamine action in the dorso-lateral striatum following electroconvulsive stimuli. In addition, decreased tachykinin NK_1 receptor mRNA in the caudal nucleus accumbens core may be due to a localized regulation of receptor synthesis and of substance P-ergic transmission. It remains

to be examined whether this is directly related to possible changes in dopaminergic transmission in the nucleus accumbens.

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