Reserpine increases proenkephalin mRNA content in rat corpus striatum

ZHENG Min[1], YANG Sheng-Li, ZOU Gang (K TSOU)[2]

(Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031)

ABSTRACT Single sc of reserpine increased the proenkephalin mRNA in rat corpus striatum dose-dependently at 24 h after injection. Reserpine did not change the proenkephalin mRNA in the basal hypothalamus, pituitary, adrenal and spleen or the proopiomelanocortin mRNA in the basal hypothalamus, pituitary, adrenal and spleen. The mechanism for reserpine to increase proenkephalin mRNA in the striatum is most likely via the removal of a tonic trans-synaptic dopaminergic inhibition on the enkephalinergic neurones.

KEY WORDS reserpine; proenkephalin; messenger RNA; corpus striatum

More than 30 neuropeptides have been found in the brain[1]. Since they are synthesized as part of a large molecular weight precursor, the dynamics of their synthesis can not be readily measured with methods commonly used in the study of classical neurotransmitters. The determination of peptide content *per se* is of limited value; however, a combination of peptide content measurement and precursor mRNA quantitation may yield more informations on the rate of synthesis and utilization of the peptide[2]. For instance, electroacupuncture has been reported to elevate the contents of enkephalins[3], enkephalin precursors and processed intermediates in the corpus striatum[4], the brain region with the highest concentration of enkephalin, indicating either there was an increase in biosynthesis or a decrease in utilization of enkephalins. Recent study in our laboratory using recombinant DNA techniques showed unequivocally that the enkephalin biosynthesis in the striatum was indeed increased by electroacupuncture as it was accompanied by an acceleration of transcription of the proenkephalin gene[5]. Pharmacological manipulation with the dopamine receptor antagonist, haloperidol, was found to increase the striatal enkephalin content[6] and the proenkephalin (PE) mRNA level[7]. This finding has led to the suggestion that dopamine tonically inhibits the biosynthesis of enkephalin in the striatum[2]. Reserpine, a depletor of amines, had also been found to increase enkephalin level in the striatum[6]. It can also block the dopaminergic tone by depleting dopamine in the striatum. It is therefore of interest to see if it can also increase PE mRNA in the corpus striatum. In this paper we report that reserpine dose-dependently increases the PE mRNA quantity in the rat striatum using the recombinant DNA technique. To study if the reserpine action is region specific, we measured the PE mRNA contents in basal hypothalamus, pituitary, adrenal gland and spleen as well.

Proopiomelanocortin (POMC) mRNA contents in the same tissues were also measured for comparison.

MATERIALS AND METHODS

Treatment of rats Sprague-Dawley ♂ rats weighing 230 ± SD 20 g, supplied by the Shanghai Experimental Animal Center, were injected sc with single doses of reserpine (Red Flag Pharmaceutical Factory,

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[1] Present Address: *Laboratory for Molecular Endocrinology, Massachusetts General Hospital, and Harvard Medical School, Boston MA 02114, USA.* [2] Correspondence: ZOU Gang

Shanghai Medical University): 1 mg/kg (7 rats) and 2, 3, and 5 mg/kg (8 rats each). Control rats were injected with saline. After 24 h the rats were decapitated and tissue samples were removed within 3 min.

Preparation of the cDNA probes A radiolabeled pHPE-9 plasmid probe (kindly donated by Prof E Herbert), containing a 918 bp cDNA sequences complementary to the proenkephalin coding region of human pheochromocytoma PE mRNA, was produced by nick-translation[8] with [α-^{32}P] dCTP (Amersham) to the final activity of 100-1000 cpm/g. A radio-labeled ME 150 plasmid probe (gift of Prof E Herbert), containing a 144 bp cDNA sequences complementary to the β-lipotropin coding region of mouse POMC mRNA was produced similarly. The ^{32}P labeled probes were purified from the nick-translation reaction solution[9].

Dot-blot hybridization The RNA samples were denatured with 2/5 volume of 15×SSC (NaCl 2.25 mol/L: sodium citrate 225 mmol/L) and 3/5 volume of formaldehyde (37%)at 60℃ for 10-15 min. Serial dilution was made with 15×SSC. Duplicates of 50 μl of each dilution were spotted on a nitrocellulose sheet (BA 85, 0.45 μm, Schleicher & Schull, pre-equilibrated in 15×SSC) in a 96 well dot-blot apparatus (BioRad). The quantity of RNA spotted was total RNA. RNA preparations (20 μg each) of 4 experiments were spotted on the same nitrocellose paper and hybridized with the same ^{32}P labeled probe. The samples were dried on the nitrocellulose sheet by applying negative pressure to the apparatus. The nitrocellulose sheet was then baked for 90 min at 80℃ *in vacuo*. Pre-hybridization, hybridization and wash were performed[10]. The washed nitrocellulose filter was exposed to a X-ray film (Shanghai Photosensitive Film Factory) for 4 h at −70℃. The autoradiograms obtained were scanned with a Shimadzu CS-930 scanning densitometer. Number above each peak represents the area under the peak. Relative density is defined as 1/100 of the actual area under each peak.

RESULTS AND DISCUSSION

With molecular hybridization techniques we studied the distributions of the PE mRNA and POMC mRNA in two brain regions and several tissues. PE mRNA was highly concentrated in the striatum, and was also detected in the basal hypothalamus, adenohypophysis, adrenal gland and spleen. Confirming previous reports, PE mRNA was not found in the liver. The POMC mRNA was detected in high concentration in the basal hypothalamus, and the neurointermediate lobe of the pituitary. It was detected also in the adenohypophysis and spleen, but not in the striatum, the adrenal gland and the liver (Tab 1).

Tab 1. Distribution of proenkephalin (PE) mRNA and proopiomelanocortin (POMC) mRNA after reserpine (+present, −absent, 0 no change, ↑ increase)

	Distribution		Change in level after reserpine	
	PE mRNA	POMC mRNA	PE mRNA	POMC mRNA
Anterior lobe of pituitary	+	+	0	0
Neurointermediate lobe of pituitary	−	+	0	0
Basal hypothalamus	+	+	0	0
Striatum	+	−	↑	0
Adrenal gland	+	−	0	0
Spleen	+	+	0	0

Reserpine was injected sc at single doses of 1, 2, 3, and 5 mg/kg. A prominent and dose-dependent increase in PE mRNA was found in the striatum (Fig 1). No significant change in the PE mRNA level was found in the anterior lobe and neurointermediate lobe of the pituitary, adrenal gland, basal hypothalamus, or spleen. The POMC mRNA level was not affected by reserpine treatment in these brain regions or tissues we studied (Tab 1).

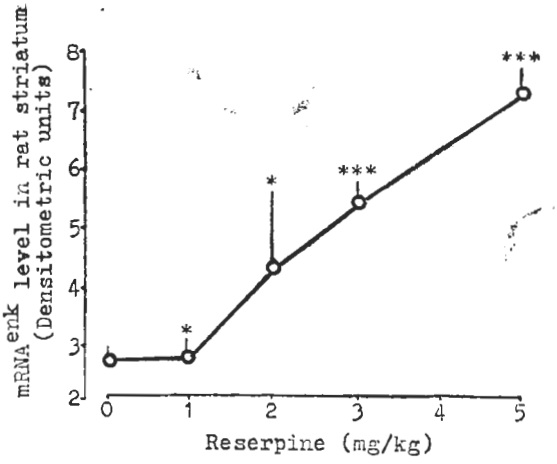

Fig 1. Increase of corpus striatal PE mRNA 24 h after sc reserpine. 4 experiments each containing 7 or 8 rats. $\bar{x}\pm SD$, *p>0.05, ***p<0.01

After the completion of our work a report by Mocchetti *et al*[11] appeared with essentially similar results. However, in contrast to our experiments, they injected sc reserpine 2 mg/kg daily × 2 d and observed selective increase of PE mRNA level in the striatum on d 3–5 after the last sc. In our experiment, the increase in PE mRNA appeared earlier, i e, 24 h after a single sc of 2 mg/kg. Since the abundant proenkephalin-derived peptides in the adrenal gland coexist with catecholamines in the medullary chromaffian granules, whereas dopamine exists presynaptically to the striatal enkephalinergic neurones, and the fact that the PE mRNA in the adrenal gland was not altered by reserpine, the increase of PE mRNA in the striatum by reserpine is most likely due to a trans-synaptic mechanism induced by the removal of dopaminergic inhibition. From our results, it may be concluded that the reserpine increase of enkephalin biosynthesis involves an acceleration of transcription of the proenkephalin gene. However, the increase in PE mRNA may also be explained by an increase in its stability due to a decreased degradation.

The finding of the presence of PE mRNA in spleen is the first time in the literature and deserves further study.

REFERENCES

Krieger DT. An overview of neuropeptides; In: Martin JB, Barchas JD, eds. *Neuropeptides in neurologic and psychiatric diseases*. NY: Raven Press, 1986 : 1-32

2 Schwartz IP, Costa E. Hybridization approach to the study of neuropeptides. *Annu Rev Neurosci* 1986; 9 : 277

3 Zou G (K Tsou), Yi QC, Wu SX, *et al*. Enkephalin involvement in acupuncture analgesia-Radioimmunoassay. *Sci Sin* 1980; 23 : 1197

4 Ding XH, Ji XQ, Zou G(K Tsou). Dynamic changes in the levels of enkephalin precursors, processed intermediates and leu-enkephalin in rat caudate nucleus following electroacupuncture. *Chin J Physiol Sci* 1986; 2 : 42

5 Zheng M, Yang SL, Zou G (K Tsou). Electroacupuncture markedly increases proenkephalin mRNA in rat striatum and pituitary. *Sci Sin* [B] 1988; 31 : 81

6 Hong JS, Yang HYT, Gillin JC, Fratta W, Costa E. Participation of [Met^5]-enkephalin in the action of antipsychotic drugs. In: Usdin E, Bunney WE Jr, Kline NS, eds. *Endorphins in mental health research*. London: Macmillan Press, 1979: 105-14

7 Tang F, Costa E, Schwartz JP. Increase of proenkephalin mRNA and enkephalin content of rat striatum after daily injection of haloperidol for 2 to 3 weeks. *Proc Natl Acad Sci USA* 1983; 80 : 3841

8 Rigby PWJ, Dieckmann M, Rhodes C, Berg P. Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. *J Mol Biol* 1977; 113 : 237

9 Zheng M, Yang SL, Zou G (K Tsou). A simplified method for the recovery of [^{32}P] DNA after nick translation. *Prog Biochem Biophys*. 1986; 13 : 77

10 Thomas PS. Hybridization of denatured RNA transfered or dotted to nitrocellulose paper. *Methods Enzymol* 1983; 100 (Part B) : 255

11 Mocchetti I, Guidotti A, Schwartz JP, Costa E. Reserpine changes the dynamic state of enkephalin stores in rat striatum and adrenal medulla by different mechanisms. *J Neurosci* 1985; 5 : 3379

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利血平增加大鼠纹状体脑啡肽原mRNA含量

郑　珉、杨胜利、邹　冈　（中国科学院上海药物研究所，上海 200031）

提要　单次sc利血平24 h后增加大鼠纹状体的脑啡肽原mRNA含量，这一效应呈剂量反应关系，1 mg/kg无作用，2，3和5 mg/kg效应按剂量增大。利血平不影响底下丘脑、垂体、肾上腺和脾脏中的脑啡肽原mRNA含量和底下丘脑、垂体和脾脏中的阿黑皮原mRNA含量。利血平可能是去除跨越突触的多巴胺对纹状体脑啡肽能神经原的紧张性抑制而起此效应的。

关键词　利血平；脑啡肽原mRNA；纹状体

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