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Autotomy and Central Nervous System Neuropeptides After Section of the Sciatic Nerve in Rats of Different Strains

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PANERAI, A E , P SACERDOTE, A BRINI, M BIANCHI AND P MANTEGAZZA *Autotomy and central nervous system neuropeptides after section of the sciatic nerve in rats of different strains* PHARMACOL BIOCHEM BEHAV **28**(3) 385–388, 1987 —Autotomy and the concentrations of beta-endorphin and Met-enkephalin in brain areas and the spinal cord were measured in Sprague Dawley, Wistar and Wistar Lewis rats thirty-five days after the section of one sciatic nerve As expected, autotomy developed in Sprague Dawley and Wistar, but not in Wistar Lewis rats In the Wistar Lewis, brain and spinal cord concentrations of Met-enkephalin increased, beta-endorphin concentrations were unchanged In Wistar and Sprague Dawley rats the increase of Met-enkephalin was accompanied by a decrease of beta-endorphin Administration of chlomipramine, a drug usually employed in the treatment of deafferentation pain, normalized the concentrations of beta-endorphin in the Sprague Dawley and Wistar rats, and avoided the development of autotomy, while Met-enkephalin concentrations did never change The data presented suggest a possible correlation between beta-endorphin and autotomy

Beta-endorphin Met-enkephalin Deafferentation Autotomy Chlomipramine

IT is well known that the lesion of peripheral nerves induces, in the experimental animal, neurophysiological changes such as an extension of the sensitive fields, or behavioral changes such as self mutilation (autotomy) of the body region affected by the lesion [1, 4, 5]

Moreover, we have recently observed (Panerai *et al* , in preparation) that deafferentation of sensitive or mixed peripheral nerves results in a decrease of beta-endorphin (BE) in brain areas of Sprague Dawley (SD) rats, and in an increase of Met-enkephalin (ME) in the brain and the spinal cord With the aim of better understanding the possible correlation between autotomy and central nervous system neurochemical changes, we measured autotomy, and the brain and spinal cord concentrations of beta-endorphin (BE) and Met-enkephalin (ME) in three different strains of rats SD, Wistar (WS) and Wistar Lewis (WL), after section of one sciatic nerve It has been shown in fact that while autotomy is frequent in the SD and WS rats [4,5], it is absent in animals of the WL strain [3]

METHOD

Male CD Sprague Dawley, Wistar, and Wistar Lewis rats, 150–200 g b wt. (Charles River, Calco, Italy), eight in each experimental group, were used in this study. Rats were housed at 22±2°C, with a 10 14 hr light dark cycle and had water and dry pellets ad lib

Animals were anesthetized with pentobarbital (40 mg/kg IP) and underwent either the section of one centimeter of the right sciatic nerve starting from its point of entrance in the spinal column, or sham operation consisting in the exposure of the sciatic nerve not followed by its section

Rats were observed daily and paws controlled for autotomy. This was considered to be present in its minimal degree when nails were bleeding. Autotomy was not graded, but only an all or nothing judgment expressed.

On day 35 after surgery, rats were killed by microwave irradiation with a method previously described and validated [7] The hypothalamus, hindbrain, and midbrain were dissected according to Glowinski [2], and the spinal cord was dissected into the cervical (C1–C7), thoracic (T1–T12) and lumbosacral (L1–S5) tracts

The tissues were homogenized in 2 ml 1 N acetic acid [7] and centrifuged, the pellet was used for protein determination [6], while the supernatant was frozen until radioimmunoassays were performed. Radioimmunoassay methods have been previously described in detail [7,8]. All antibodies were obtained in our laboratory, the antibody used for BE

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TABLE 1

CONCENTRATIONS OF β-ENDORPHIN AND MET-ENKEPHALIN IN THE CNS OF SPRAGUE DAWLEY, WISTAR AND WISTAR LEWIS RATS 35 DAYS AFTER THE SECTION OF THE RIGHT SCIATIC NERVE

	Sprague Dawley			
	Met-Enkephalin[a]		β-Endorphin	
	Sham	Deafferentated	Sham	Deafferentated
Hypothalamus	4 0±1 0[b]	25 ±5†	98 ±15	11 ±7†
Midbrain	1 5±0 5	8 2±1 0†	1 8± 0 4	0 4±0 1†
Hindbrain	2 2±0 5	4 0±0 5*	0 8± 0 2	0 2±0 05†
Cervical spinal cord	1 2±0 5	2 4±0 6	ND	ND
Thoracic spinal cord	1 3±0 6	2 5±0 5	ND	ND
Lumbosacral spinal cord	1 2±0 7	14 ±1 2†	ND	ND

[a]=all values are ng/mg protein
[b]=mean±SD
ND=not detectable
*=$p<0$ 05, †=$p<0$ 01 vs sham

TABLE 2

CONCENTRATIONS OF β-ENDORPHIN AND MET-ENKEPHALIN IN THE CNS OF SPRAGUE DAWLEY, WISTAR AND WISTAR LEWIS RATS AFTER SECTION IN THE SCIATIC NERVE AND TREATMENT WITH SALINE OR CHLOMIPRAMINE (CIM)

	Sprague Dawley			
	Met-Enkephalin[a]		β-Endorphin	
	Sham	CIM	Sham	CIM
Hypothalamus	23 ±2 0[b]	21 ±6	15 ±10	88 ±4†
Midbrain	7 5±0 4	7 2±1 5	0 2± 0 2	2 3±0 3*
Hindbrain	4 1±0 4	3 6±0 1	0 1± 0 04	0 6±0 05
Cervical spinal cord	1 0±0 5	1 5±0 9	ND	ND
Thoracic spinal cord	1 4±0 9	2 4±0 9	ND	ND
Lumbar spinal cord	13 ±0 8	12 5±0 5	ND	ND

[a]=all values are ng/mg protein
[b]=mean±SD
ND=not detectable
*=$p<0$ 05, †=$p<0$ 01 vs sham

shows 100% cross reactivity with beta-lipotropin and POMC, the antibody toward ME shows 1% cross reactivity with Leu-enkephalin and 100% with proenkephalin-A.

Starting the day of surgery, other rats of each strain were treated for 35 days with either saline or the tricyclic antidepressant chlomipramine (CIM, Ciba-Geigy, Origgio, Italy), twice daily at the dose of 5 0 mg/kg intraperitoneally

Statistical analysis of results was performed by the Student's *t*-test for comparisons within sham operated and deafferentated rats of the same strain

RESULTS

Table 1 shows the effects of the section of the sciatic nerve on BE and ME concentrations in the hypothalamus, midbrain, hindbrain, and the different tracts of the spinal cord of SD, WL and WS rats, 35 days after surgery BE concentrations decrease significantly in the brain areas of SD and WS rats, while remaining unchanged in the WL rats In contrast, ME concentrations constantly increase in the brain areas of the rats of the three strains. Deafferentation also induced an increase of ME concentrations in the spinal cord of rats of all strains, and the regional distribution of the changes we observed seems to reflect the site of entrance of sensory efferents in the spinal cord As far as the treatment with CIM is concerned, this normalized BE concentrations in SD and WS rats, and it did not modify BE in WL rats, as it is shown in Table 2. ME concentrations were never affected by the treatment with CIM in any experimental group.

Autotomy was observed only in SD and WS rats, while it was always absent in WL Moreover, it was absent in SD and WS rats that underwent treatment with CIM

TABLE 1
(Continued)

Wistar				Wistar Lewis			
Met-Enkephalin		β-Endorphin		Met-Enkephalin		β-Endorphin	
Sham	Deafferentated	Sham	Deafferentated	Sham	Deafferentated	Sham	Deafferentated
4 2±0 9	21 ±4†	96 ±12	10 ±6†	3 8±1 2	23 4±2 3†	90 ±10	84 ±12
1 6±0 6	7 4±0 8†	1 4± 0 2	0 3±0 05†	1 2±0 5	7 5±0 8†	1 6± 0 5	1 5± 0 8
1 8±0 4	4 3±0 6†	0 1± 0 5	0 2±0 05†	2 0±0 8	4 2±0 05*	0 7± 0 2	0 8± 0 1
1 1±0 4	2 2±0 4	ND	ND	1 1±0 4	2 0±0 6	ND	ND
1 2±0 6	2 5±0 4	ND	ND	1 0±0 3	1 5±0 3	ND	ND
1 2±0 8	12 ±1 0†	ND	ND	1 2±0 6	11 1±0 2†	ND	ND

TABLE 2
(Continued)

Wistar				Wistar Lewis			
Met-Enkephalin		β-Endorphin		Met-Enkephalin		β-Endorphin	
Sham	CIM	Sham	CIM	Sham	CIM	Sham	CIM
20 ±1	18 ±2	11 ±11	90 ±15†	23 ±4	22 ±6	88 ±10	89 ±6
7 3±0 3	7 9±0 9	0 1± 0 1	1 8±0 1†	6 8±1 3	7 4±1 2	1 8± 0 3	1 6±0 2
4 1±0 2	3 2±0 4	0 2± 0 1	0 7±0 05	1 7±0 1	3 4±0 3	0 8± 0 3	0 7±0 1
1 4±0 6	1 6±0 2	ND	ND	1 3±0 6	1 8±0 6	ND	ND
1 8±0 6	1 5±0 5	ND	ND	1 2±0 4	1 5±0 2	ND	ND
13 ±0 4	15 0±0 6	ND	ND	11 ±2	11 0±1 2	ND	ND

DISCUSSION

The data presented confirm and expand the previous demonstration of BE and ME changes in the central nervous system of animals that underwent the deafferentation of peripheral nerves. Moreover, the data obtained in SD and WS are consistent with the effect previously described of CIM in increasing BE concentrations [12].

The overall evaluation of the correlation between the presence of autotomy, and BE and ME concentrations in brain areas and the spinal cord of SD, WS and WL rats after deafferentation alone or with the concomitant treatment with CIM, suggests that BE might be an important substrate for the development of autotomy. In fact, autotomy is present in SD and WS rats, in which brain BE decreases after deafferentation, while neither autotomy nor the decrease of brain BE are present in WL rats. Moreover, autotomy is absent in SD and WS rats after treatment with the serotoninergic drug chlomipramine, that normalizes the BE concentrations decreased by the deafferentation. Other data from the literature are consistent with this role for BE It is known in fact that autotomy develops also in capsaicin treated rats [11], and in rats with experimental arthritis [11], two experimental models in which BE in brain areas is greatly decreased [9]

The data presented suggest that WL rats might bear significant neurochemical differences from other strains of rats such as the SD or the WS It appears in fact that although basal BE concentrations are similar in WL, SD and WS rats, these are not increased in WL by stimulation of the serotoninergic system, as it happens in the other two strains [12] The derangement of the BEergic system might be one important feature of WL rats, and deserves further studies, directed to evidentiate other neurochemical differences involving peptides or neurotransmitters.

In conclusion, from the consideration of the results, and the data reported from the literature in different experimental conditions, it appears that the decrease of BE might be responsible for, or at least facilitate, the development of autotomy

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