6. H. U. Bergmeyer and H. O. Beutler, in: *Methods of Enzymatic Analysis*, Vol. 8 (H. U. Bergmeyer, ed.), Weinheim (1984), pp. 454-461.
7. V. Felipo, E. Grau, M. D. Minana, and S. Grisolia, in: *Cirrhosis, Hyperammonemia and Hepatic Encephalopathy* (S. Grisolia *et al.*, eds.), New York (1994), pp. 83-93.
8. V. Felipo, M. D. Minana, R. Wallace, and S. Grisolia, *J. Clin. Nutr. Gastroenterol.*, **5**, № 3, 165-169 (1990).
9. E. A. Kosenko, Yu. G. Kaminsky, V. Felipo, *et al.*, *Biochim. Biophys. Acta*, **1180**, № 1, 321-326 (1993).
10. G. Marcaida, V. Felipo, C. Hermenegildo, *et al.*, *FEBS Lett.*, **296**, № 1, 67-68 (1992).
11. F. Noll, in: *Methods of Enzymatic Analysis*, Vol. 6 (H. U. Bergmeyer, ed.), Weinheim (1984), pp. 582-589.
12. N. Seiler, S. Sarhan, B. Knoedgen, *et al.*, *Pharmacol. Toxicol.*, **72**, № 1, 116-123 (1993).
13. K. V. Toyka, in: *The Cholinergic Synapse*, Berlin (1988), pp. 697-724.
14. T. R. Ulshafer, *J. Lab. Clin. Med.*, **52**, № 5, 718-723 (1958).
15. R. P. Wilson, M. F. Muhrer, and R. A. Bloomfield, *Comp. Biochem. Physiol.*, **25**, № 1, 295-301 (1968).

Enkephalinase Activity in Various Brain Structures of Naloxone-Treated and Morphine-Insensitive Rats

L. F. Panchenko, V. Yu. Baronets, N. N. Terebilina, V. V. Aristova,* S. V. Litvinova,* and L. V. Kalyuzhnyi**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 120, № 11, pp. 492-494, November, 1995
Original article submitted November 21, 1994

Morphine injected subcutaneously in a dose of 2 mg/kg body weight exerted an analgesic effect in some Wistar rats (morphine-sensitive animals), as was indicated by a significantly prolonged latency of the tail-flick response, but failed to produce analgesia in others (morphine-insensitive animals). In morphine-sensitive rats, the striatum had the highest enkephalinase A activity, followed in decreasing order by the mesencephalon, hippocampus, pons, cortex, medulla oblongata, and hypothalamus. Thirty minutes after intraperitoneal administration of naloxone (0.3 mg/kg body weight) to morphine-sensitive rats, enkephalinase activity fell significantly in the hippocampus, striatum, and cortex, remained unchanged in the pons and medulla oblongata, and rose significantly in the mesencephalon and insignificantly in the hypothalamus; generally similar differences in enkephalinase activity from naloxone-untreated morphine-sensitive rats were recorded in the brain structures of morphine-insensitive rats given saline instead of naloxone.

Key Words: *morphine; sensitivity; enkephalinase; naloxone; nociception; analgesia*

Naloxone inhibits the analgesic effect of morphine [7] mediated by increased release of endogenous opioids [4]. However, individual animals, and up to 30% of rats in particular, are insensitive to morphine and to acupuncture [5,6]. Such rats were found to contain lowered levels of endogenous opioids [6], as were morphine-sensitive (MS) rats following administration of naloxone alone [3]. These findings may be accounted for either by reduced release of endogenous opioids or by heightened activity of the endopeptidases that inactivate them [3]. d-Phenylalanine, which inhibits enkephalinase A, wes shown to restore the analgesic effect of morphine in morphine-insensitive (MI) and acupuncture-insensitive animals [2,6].

It was therefore decided to compare enkephalinase A activity in different parts of the brain in MI and MS rats and in naloxone-treated and -untreated MS rats.

State Center of Addiction, Ministry of Health and Medical Industry of the Russian Federation, Moscow; *Department of Higher Nervous Activity, Moscow State University; **P. K. Anokhin Institute of Normal Physiology, Moscow (Presented by K. V. Sudakov, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0011-1114$12.50

MATERIALS AND METHODS

A total of 20 male Wistar rats weighing 250-300 g were used. Alterations in nociception were estimated using the previously described procedure [2] of the tail-flick test, in which the latency of the tail-flick response to a nociceptive thermal stimulus was recorded with an automatic analgesimeter (Ugo Basile).

Enkephalinase A activity in different brain structures was measured as previously described [1].

Morphine hydrochloride was injected subcutaneously in a dose of 2 mg/kg and naloxone (Sigma) intraperitoneally in a dose of 0.3 mg/kg. All rats were decapitated 30 min after the administration of naloxone or of saline in the same volume and by the same route.

The results were treated statistically by the unpaired Student's test.

RESULTS

In 15 of the 20 rats tested, morphine prolonged the latency of their tail-flick response to the nociceptive thermal stimulus from the initial (preinjection) value of 16±0.2 sec to 40 sec 10-15 min postinjection, and the analgesic effect persisted for 30-40 min (MS animals). In the remaining rats, with an initial tail-flick latency of 16.3±0.3 sec, morphine administration was not followed by a significant prolongation of the latency, which increased only to 16.9±0.4 sec 10-15 min postinjection (MI animals). In 5 of the MS rats with an initial tail-flick latency of 17.6±0.4 sec, naloxone produced a hyperalgesic effect 10-15 min postinjection, reducing the latency to 9.3±0.4 sec ($p<0.001$). The remaining 15 rats, which received saline in the same volume, did not show a significant change in the response latency (this increased to 18.2±0.4 sec).

Enkephalinase A activity in the MS rats varied from one brain structure to another, being highest in the striatum and progressively lower in the mesencephalon, hippocampus, pons, cortex, medulla oblongata, and hypothalamus (Table 1). After naloxone administration to MS rats, the highest enkephalinase A activity was shown by the mesencephalon, followed by the pons, striatum, medulla, hypothalamus and hippocampus, and cortex, in that order. As a result of naloxone, enkephalinase A activity fell significantly in the hippocampus, striatum, and cortex, remained virtually unchanged in the pons and medulla, and rose significantly (by 20%) in the mesencephalon and insignificantly (by 15%) in the hypothalamus.

In the saline-injected MI rats, the brain structures studied can be arranged (Table 1) in order of decreasing enkephalinase A activity as follows: mesencephalon, pons, striatum, hippocampus, medulla and hypothalamus, and cortex, i.e., in the same order as in the naloxone-injected MS rats with the exception of the hippocampus. Differences in enkephalinase activity between the MI rats and the naloxone-untreated MS rats were also similar to, though less marked than, those between the latter rats and the naloxone-treated MS animals. Thus, the enkephalinase activity of MI rats was significantly lower in the hippocampus, striatum, and cortex (on average by 52, 35, and 45%, respectively), insignificantly lower in the medulla (by 36% on average), nearly the same in the pons, and insignificantly higher in the mesencephalon (by 11%) and hypothalamus (by 8%).

The results presented above show that enkephalinase A activity in MS rats varied from one brain region to another and that the changes in this activity in MI rats were similar to those recorded for MS rats after naloxone administration.

The levels of enkephalins are known to be unequal in different structures of the rat brain, being high and more or less the same in the hypothalamus, mesencephalon, and medulla and 1.5 to 2 times lower in the cortex and hippocampus [3]. In the present study, enkephalinase activity in the con-

TABLE 1. Enkephalinase Levels in Different Brain Structures of Morphine–Insensitive (MI) and Morphine–Sensitive (MS) Rats (pmol/mg protein/min)

Brain structure	MS rats (n=15)	MI rats (n=5)	MS rats injected with naloxone (n=5)
Pons	41.20±3.40	42.37±5.90	43.68±4.50
Hippocampus	52.45±5.40	25.22±3.3**	20.25±3.00***
Striatum	64.61±4.54	42.31±2.45**	34.79±1.94***
Hypothalamus	17.36±1.78	18.73±1.78	20.41±2.14
Mesencephalon	61.70±3.20	68.31±6.60	76.60±4.60*
Medulla oblongata	29.44±3.20	18.83±8.50	29.33±6.50
Cortex	31.37±1.97	17.15±1.08**	17.19±0.41***

Note. *$p<0.05$, **$p<0.01$, ***$p<0.001$ in comparison with MS rats.

trol (saline-injected) MS rats was found to be highest in the striatum, mesencephalon, and hippocampus; lower in the cortex and medulla; and lowest in the hypothalamus. Among the brain parts studied, the major role in mediating the analgesic effects of morphine and acupuncture is played by key structures of the endogenous antinociceptive system such as the central gray matter within the mesencephalon (higher values of enkephalinase activity could probably be obtained if it were measured in this matter separately) and the hypothalamus. Acupuncture-induced analgesia has been shown to be associated with considerable rises of enkephalins in the hypothalamus, central gray matter, and striatum and smaller rises in the cortex, hippocampus, and thalamus [8,10]. The ratios that exist between enkephalin levels and enkephalinase activity in the hypothalamus, central gray matter, and thalamus probably account for the high levels of enkephalins *in situ* and for their release in response to morphine [4] or acupuncture [8,10] with a resultant blockade of the nociceptive impulse traffic.

Morphine and acupuncture were previously reported not to produce an analgesic effect in MI or in MS rats injected with naloxone [5,6]. In such animals, the ratios between enkephalin levels and enkephalinase activity in the key structures of the endogenous antinociceptive system appear to be substantially altered. Rats injected with naloxone at 5 mg/kg body weight were found to have reduced enkephalin levels in virtually all of the brain structures mentioned above [3]. In the present study, naloxone administered to MS rats at 0.3 mg/kg, i.e., in a dose that blocks the analgesic action of morphine [7], lowered enkephalinase activity in the hippocampus, striatum, and cortex, which, together with the decline of enkephalins, probably preserved the former balance between enkephalins and enkephalinase activity. On the other hand, enkephalinase activity remained unchanged or even rose in the key structures of the endogenous antinociceptive system (mesencephalon, hypothalamus, and medulla), and this undoubtedly accentuated the differences in the ratio between enkephalin and enkephalinase levels. Similar, though less marked, differences were observed for MI rats. The high enkephalinase activity and low enkephalin levels [3] in the key structures of the endogenous antinociceptive system are evidently responsible for the inadequate release of endogenous opioids or for their rapid inactivation by endopeptidases in MI rats exposed to morphine or acupuncture and, consequently, for the failure of these to induce analgesia. This possibility is supported by the finding that administration of the enkephalinase inhibitor d-phenylalanine restored the analgetic effects of morphine and acupuncture in MI rats [2,6] and tripled the level of enkephalins in the hypothalamus [9].

It may be concluded that MI rats and naloxone-injected MS rats develop similar changes in enkephalinase activity in practically all of the brain structures examined, but that this activity is higher in some structures and lower in others, which results in unequal ratios of enkephalinase activity to endogenous enkephalin levels in different structures. One reason for the failure of morphine and acupuncture to produce analgesia in MI rats may be elevated enkephalinase activity and a correspondingly reduced release of endogenous enkephalins in the mesencephalic central gray matter and hypothalamus, i.e., in the key structures of the endogenous antinociceptive system.

REFERENCES

1. N. A. Belyaev, E. F. Kolesanova, V. Yu. Baronets, *et al.*, *Biokhimiya*, **55**, 1778-1785 (1990).
2. S. V. Litvinova, A. Yu. Kozlov, and L. V. Kalyuzhnyi, *Byull. Eksp. Biol. Med.*, **116**, № 7, 54-56 (1993).
3. K. Gudehithlu, G. Tejwani, and H. Bhargava, *Brain Res.*, **553**, 284-290 (1991).
4. J. Kachur, B. Rosemond, S. Welch, *et al.*, *Life Sci.*, **37**, 2549-2555 (1985).
5. L. Kalyuzhnyi, V. Iasnetsov, and L. Vyklicky, *Physiol. Bochemoslov.*, **34**, № 1, 1-10 (1985).
6. C. Takeshigi, in: *Synaptic Transmission in Acupuncture Analgesia*, Showa Univ. (1992), p. 334.
7. A. Vacarino, R. Tasker, and R. Melzack, *Pain*, **36**, 103-109 (1989).
8. C. Xiaoding, X. Shaofen, H. Liafang, *et al.*, *Chen. Tzu. Yen. Chiu. [Acupuncture Research]*, **14**, № 1-2, 199-202 (1989).
9. H. Yuan and J. Han, *Acta Physiol. Sin.*, **37**, № 3, 265-273 (1985).
10. R. Zou, Q. Yi, S. Wu, *et al.*, *Sci. Sin. [B]*, **23**, № 9, 1197-1207 (1980).