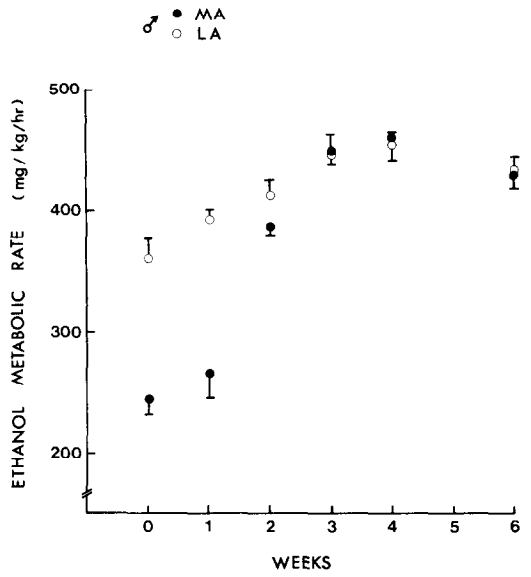

Fig. 1. Ethanol metabolic rates for MA and LA male rats determined during the course of chronic ethanol treatment. Each point represents mean ± S.E.M.; N = six to eight animals per group.

low rates, show a marked metabolic tolerance (80–100%) of the order of that reported here for MA animals [9]. However, females of the same line have high initial EMR values and show only a modest increase (30–40%) as also reported here for the LA and MA females [10].

In the spontaneously hypertensive male rats [9] and in the male Sprague–Dawley [11, 12] rates of ethanol metabolism in mature naive animals are associated with a low alcohol dehydrogenase (ADH) activity, an enzyme that, in these lines, is repressed by testosterone. It has been shown that chronic alcohol consumption leads to a reduction in testosterone levels with an associated increase in ADH activity and in the rate of ethanol metabolism. A low ADH activity has also been shown in MA males as compared to MA females by Lester *et al.* [13]. Thus, it is conceivable that the mechanism leading to a greater relative metabolic tolerance in the males of the MA line following chronic ethanol consumption may also be sex hormone dependent.

The findings reported may explain the marked differences in metabolic tolerance following chronic ethanol consumption reported by many investigators both in experimental animals and humans.

Department of Pharmacology JATINDER M. KHANNA
University of Toronto YEDY ISRAEL
Toronto, Canada M5S 1A8 HAROLD KALANT

and

Addiction Research Foundation of Ontario, Toronto, Canada M5S 2S1 JOEL M. MAYER

REFERENCES

1. K. J. Isselbacher and N. J. Greenberger, *New Engl. J. Med.* **270**, 351 (1964).
2. W. W. Westerfeld and M. P. Schulman, *J. Am. med. Ass.* **170**, 197 (1959).
3. T. E. Reed, H. Kalant, R. J. Gibbins, B. M. Kapur and J. G. Rankin, *Can. med. Ass. J.* **115**, 851 (1976).
4. C. S. Lieber, in *Metabolic Aspects of Alcohol* (Ed. C. S. Lieber), p. 11. University Park Press, Baltimore (1977).
5. J. M. Khanna and Y. Israel, in *Liver and Biliary Tract Physiology I, International Review of Physiology* (Ed. N. B. Javitt), Vol. 21, p. 284. University Park Press, Baltimore (1980).
6. E. P. Riley, E. X. Freed and D. Lester, *J. Stud. Alcohol* **37**, 1535 (1976).
7. R. D. Hawkins, H. Kalant and J. M. Khanna, *Can. J. Physiol. Pharmac.* **44**, 261 (1966).
8. J. M. Khanna and H. Kalant, *Biochem. Pharmac.* **19**, 2033 (1970).
9. G. Rachamin, J. A. MacDonald, S. Wahid, J. J. Clapp, J. M. Khanna and Y. Israel, *Biochem. J.* **186**, 483 (1980).
10. G. Rachamin, *M. Sc. Thesis*, Department of Pharmacology, University of Toronto, Toronto, Canada (1978).
11. E. Mezey, J. J. Potter, S. M. Harmon and P. D. Tsitouras, *Biochem. Pharmac.* **29**, 3175 (1980).
12. T. J. Cicero, J. D. Bernard and K. Newman, *J. Pharmac. exp. Ther.* **215**, 317 (1980).
13. D. Lester, G. W-J. Lin, N. Anandam, E. P. Riley, E. D. Worsham and E. X. Freed, in *Alcohol and Aldehyde Metabolizing Systems* (Eds. R. G. Thurman, J. C. Williamson, H. Drotte and B. Chance), Vol. 3, p. 635. Academic Press, New York (1977).

Biochemical Pharmacology, Vol. 31, No. 19, pp. 3141–3143, 1982.
Printed in Great Britain.

0006-2952/82/193141-03 $03.00/0

Effects of morphine on norepinephrine turnover in various functional regions of rat spinal cord

(*Received* 29 *January* 1982; *accepted* 16 *April* 1982)

The locus coeruleus (LC), the A5 pontine nucleus and the A1/A2 catecholaminergic nuclei provide the major noradrenergic innervation to the ventral horn, the zona intermedia, and the dorsal horn, respectively, of the spinal cord [1–4]. Recently, a number of reports have implicated spinal norepinephrine (NE) in the antinociceptive effects of morphine administered either systemically [5, 6] or into discrete brain nuclei [7]. Moreoever, NE, when administered intrathecally to the spinal cord, causes analgesia [8, 9]. On the basis of the above neuroanatomical and pharmacological considerations, one would predict that the most likely site for the interaction of morphine with spinal noradrenergic transmission would be the dorsal horn. We now report, on the basis of a detailed investigation, that

systemically administered morphine produces a very selective increase in the turnover rate of norepinephrine (TR_{NE}) in the zona intermedia (ZI) but not in the dorsal horn or the ventral horn of the spinal cord. The results suggest that morphine produces an activation of those neurons in A5 which innervate the preganglionic sympathetic neurons in the ZI of the thoracic and upper lumbar regions of the spinal cord of rat [3]. The results support a recent report that the spinal antinociceptive effect of NE, mediated via neurons in the dorsal horn, does not exhibit cross-tolerance with morphine and is not blocked by naloxone [8].

In the first set of experiments, male Sprague–Dawley rats, 200–250 g, were injected subcutaneously with saline or morphine sulfate (10 mg/kg) or naloxone (2 mg/kg) plus morphine. Sixty minutes after morphine administration, the animal was decapitated, and the whole spinal cord was rapidly removed. The cord was subsequently carefully dissected into the five functional regions to be analyzed. The cord was placed on its ventral surface on a plate of glass, frozen on solid CO_2, and divided into cervical, thoracic and lumbar regions. For the dissection of the dorsal and ventral horns of the cervical or lumbar cord, 0.5 mm thick vertical cuts were made on either side of the cord, which was then placed on its side. These cuts exposed a gray line running longitudinally along the sides of the trimmed cord. The dorsal and vertical halves of the cord were then separated by cutting vertically through this longitudinal gray line. For the zona intermedia, the thoracic cord was similarly trimmed and laid on its side. Similar vertical cuts were made at either side of the exposed gray line separating the dorsal and ventral horn respectively. The zona intermedia was the strip of gray matter that remained. Dopamine (DA), NE, dihydroxyphenylacetic acid (DOPAC) and methoxyhydroxyphenylglycol (MHPG) were analyzed by mass fragmentography using methods previously described in detail [10, 11].

In the second set of experiments, rats were treated with α-methyl-*p*-tyrosine (α-MPT) (250 mg/kg, i.v.) at 0 min. α-MPT is known to inhibit tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of catecholamines [11]. At 60 min the α-MPT-treated animals were divided into two groups and given either morphine (10 mg/kg, s.c.) or saline. The concentration of NE in these animals was assessed at 120 min. The rats of a second group were given either morphine or saline at 0 min, and killed at 60 min. The NE values obtained in these two latter groups were statistically identical (Table 1). The saline-treated group was therefore used as a control for the α-MPT plus saline group. Similarly, the morphine-treated group was used as the control for the α-MPT plus morphine group.

The results in Table 2 show that only the concentration of MHPG in the ZI was increased significantly after morphine. This effect was reversed by naloxone. The results illustrated in Fig. 1 support the idea that the selective increase of MHPG in ZI is indicative of an increase in the TR_{NE} in this region of the cord.

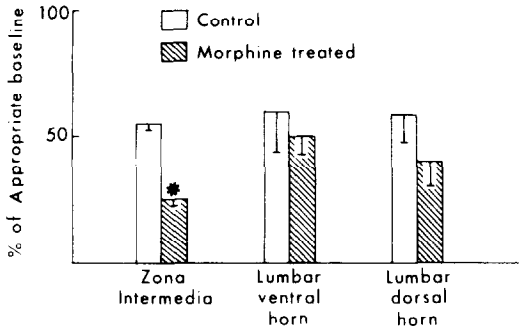

Fig. 1. Effect of α-MPT on the rate of NE depletion by morphine in various regions of spinal cord. Rats were injected with α-methyl-*p*-tyrosine (α-MPT) (250 mg/kg, i.v.) at 0 min and then with morphine sulfate (morphine-treated) (10 mg/kg, s.c.) or saline (control) at 60 min, and killed at 120 min. Animals for baseline values were injected with morphine or saline at 0 min and killed at 60 min. NE values in the α-MPT-treated animals are expressed as a percentage of the appropriate baseline control (i.e. saline or morphine-treated rats not given α-MPT). See Table 1 for baseline values. Key: (*) $P < 0.01$.

In the α-MPT/morphine-treated rats, there was a significant increase in the rate of depletion of NA only in the ZI (Fig. 1). Morphine did not affect the rate of depletion of NA in either the dorsal horn or the ventral horn of the lumbar spinal cord. Both sets of results taken together indicate strongly that morphine produced a selective increase in the TR_{NE} only in the ZI of the thoracic cord.

These results indicate that, in the cord, the only site of interaction of morphine with spinal noradrenergic mechanism was in the ZI, the main site of origin of the preganglionic sympathetic neurons. This is the region of the spinal cord with the densest noradrenergic innervation [4]. The results provide no indication of the exact mechanism or initial site of action of morphine. Increases in transmitter turnover rate are usually indicative of increased neuronal activity. Hence, one would predict that morphine selectively activates either the cell bodies of those noradrenergic neurons in A5 which project to the zona intermedia or the terminals of these neurons in the cord [3]. The fact that the density of opiate receptors is not great either in the A5 nucleus or in the zona intermedia of the cord [12] suggests that one is dealing with an indirect effect of morphine, whose net, final outcome is activation of A5 noradrenergic neurons. There was no significant effect of morphine on the spinal dopaminergic neurons in any of the regions studied.

Our results, by demonstrating that morphine does not affect NE metabolism in the dorsal horn, lend support to a recent report that the spinal antinociceptive effect of NE

Table 1. Effect of morphine or saline on the levels of norepinephrine in various regions of rat spinal cord*

Treatment	NE [ng/mg protein ± S.E.M. (N = 8)]		
	Lumbar dorsal horn	Lumbar ventral horn	Thoracic zona intermedia
Saline	8.5 ± 0.8	7.0 ± 1.1	10.0 ± 1.0
Morphine	7.6 ± 0.6	7.5 ± 0.6	8.1 ± 3.0

* Rats were injected with either saline or morphine at 0 min and killed at 60 min. NE was analyzed mass fragmentographically. The values for the saline and morphine-treated groups were statistically identical. Therefore, these results were used as the control values in computing the data presented in Fig. 1.

Table 2. Effect of morphine on the metabolism of dopamine and noradrenaline in the spinal cord of rat*

Treatment	NA	MHPG	DA	DOPAC
	[ng/mg protein ± S.E.M. (N = 8)]			
Cervical				
Dorsal horn				
Control	6.1 ± 0.6	1.5 ± 0.1	0.47 ± 0.07	0.49 ± 0.06
Morphine	6.7 ± 0.2	1.7 ± 0.1	0.47 ± 0.02	0.67 ± 0.05
Morphine + naloxone	8.4 ± 0.2	1.7 ± 0.1	0.58 ± 0.07	0.44 ± 0.01
Ventral horn				
Control	7.0 ± 0.7	1.6 ± 0.1	0.33 ± 0.09	0.36 ± 0.06
Morphine	6.3 ± 0.4	1.8 ± 0.2	0.21 ± 0.02	0.48 ± 0.07
Morphine + naloxone	6.4 ± 0.6	1.4 ± 0.1	0.22 ± 0.03	0.50 ± 0.11
Thoracic				
Zona intermedia				
Control	8.4 ± 0.4	1.5 ± 0.1	0.66 ± 0.04	0.61 ± 0.05
Morphine	7.7 ± 0.6	2.2 ± 0.1†	0.51 ± 0.03	0.64 ± 0.09
Morphine + naloxone	9.3 ± 0.8	1.7 ± 0.1	0.59 ± 0.04	0.41 ± 0.11
Lumbar				
Dorsal horn				
Control	8.5 ± 0.8	1.7 ± 0.1	0.64 ± 0.06	0.74 ± 0.10
Morphine	7.6 ± 0.5	1.4 ± 0.2	0.48 ± 0.09	0.51 ± 0.04
Morphine + naloxone				
Ventral horn				
Control	6.9 ± 1.1	1.6 ± 0.2	0.68 ± 0.08	0.55 ± 0.09
Morphine	7.5 ± 0.5	1.3 ± 0.1	0.38 ± 0.07	0.53 ± 0.23
Morphine + naloxone				

* Morphine sulfate (10 mg/kg) was administered subcutaneously and rats were killed after 60 min. Naloxone (2 mg/kg) was administered subcutaneously at −2 min and 30 min, and morphine sulfate (10 mg/kg) s.c. at 0 min; rats were killed at 60 min. Catecholamines and metabolites were measured by mass fragmentography as previously described [10, 11].

† $P < 0.01$ by independent t-test.

in the dorsal horn does not exhibit cross-tolerance with morphine and is not blocked by naloxone [8]. However, it has been reported that morphine, after systemic administration, increases the normetanephrine concentration in the dorsal horn of the cord [5]. Assuming that MHPG and normetanephrine are both valid indices of NE metabolism, our results are clearly at variance with those of Shiomi and Takagi [5].

Perhaps the most interesting feature of the results is to alert us to the possibility that some effects of morphine in the central nervous system, that involve well-defined functional regions, are unrelated to its narcotic analgesic effect. This idea is in keeping with current concepts of the relatively widespread distribution of opiate peptidergic neuronal systems in the CNS. A number of these systems have been defined in both the spinal cord and the brain [12], and it is unlikely that all of them are involved with mechanism of analgesia. The data presented deal with the acute effects of morphine measured 60 min after drug injection. Therefore, it is not clear whether chronic morphine administration would affect NE metabolism in the cord differently.

Laboratory of Clinical Psychopharmacology
Division of Special Mental Health Research
Intramural Research Program
National Institute of Mental Health
Saint Elizabeth's Hospital
Washington, DC 20032, U.S.A.

FAROUK KAROUM
JOHN COMMISSIONG*
RICHARD JED WYATT

REFERENCES

1. J. W. Commissiong, S. O. Hellstrom and N. H. Neff, *Brain Res.* **148**, 207 (1978).
2. L. Nygren and L. Olson, *Brain Res.* **132**, 85 (1977).
3. A. D. Loewy, S. McKellar and C. B. Saper, *Brain Res.* **174**, 309 (1979).
4. J. W. Commissiong, *Brain Res.* **212**, 145 (1981).
5. H. Shiomi and H. Takagi, *Br. J. Pharmac.* **52**, 519 (1974).
6. H. Takagi, H. Shiomi, Y. Kuraishi, K. Fukui and H. Ueda, *Eur. J. Pharmac.* **54**, 99 (1979).
7. T. Yaksh, *Brain Res.* **160**, 180 (1979).
8. S. V. R. Reddy and T. L. Yaksh, *Brain Res.* **189**, 391 (1980).
9. P. M. Headley, A. W. Duggan and B. T. Griersmith, *Brain Res.* **145**, 185 (1978).
10. F. Karoum, J. C. Gillin, R. J. Wyatt and E. Costa, *Biomed. Mass Spectrome.* **2**, 183 (1975).
11. F. Karoum, C. Garrison, N. H. Neff and R. J. Wyatt, *J. Pharmac. exp. Ther.* **201**, 654 (1977).
12. M. Sar, K. I. E. Stumpf, R. J. Miller, K. Chang and P. Cuatrecasas, *J. comp. Neurol.* **182**, 17 (1978).

* Correspondence should be sent to J. Commissiong at his present address: Department of Physiology, McIntyre Medical Building, McGill University, 3655 Drummond St., Montreal, Quebec H3G 1Y6.