Biochemical Pharmacology, Vol. 26, pp. 2355–2358. Pergamon Press, 1977. Printed in Great Britain.

THE EFFECTS OF ACUTE AND CHRONIC ADMINISTRATION OF MORPHINE ON NOREPINEPHRINE TURNOVER IN RAT BRAIN REGIONS

MARK ROFFMAN*, GERALDINE CASSENS and JOSEPH J. SCHILDKRAUT

Department of Psychiatry, Harvard Medical School, Neuropsychopharmacology Laboratory, Massachusetts Mental Health Center, Boston, MA 02115, U.S.A.

(*Received* 1 *October* 1976; *accepted* 30 *March* 1977)

Abstract—The effects of acute and chronic administration of morphine on norepinephrine turnover in rat brain regions were assessed by measuring changes in the levels of 3-methoxy-4-hydroxyphenylglycol sulfate (MHPG-SO_4), the major metabolite of norepinephrine (NE) in rat brain. Acute administration of morphine sulfate (25 mg/kg, i.p.) significantly increased levels of MHPG-SO_4 in hypothalamus, cerebellum, brainstem and "rest of brain" but not in cortex or corpus striatum. After chronic administration of increasing doses of morphine, tolerance developed to this effect of morphine on MHPG-SO_4 levels in cerebellum, brainstem and "rest of brain", but tolerance to the morphine-induced increase in MHPG-SO_4 levels was not observed in hypothalamus. Sixteen hr after the cessation of chronic morphine administration, levels of MHPG-SO_4 were significantly reduced in hypothalamus, cerebellum and "rest of brain". These findings are discussed in relation to the regional specificity of the action of morphine on NE turnover, and the possible role of noradrenergic neurons in the reinforcing properties of morphine.

In recent years, a number of investigators have examined the effects of acute and chronic administration of morphine on the turnover of catecholamines either in whole brain or in brain regions. In contrast to the relatively consistent findings indicating that morphine increases dopamine turnover [1–6], studies of the effects of morphine on norepinephrine (NE) turnover in brain have been contradictory.

Using the rate of formation of radioactively labelled NE from labelled tyrosine as an estimate of NE turnover, some studies have reported that the acute administration of morphine increases NE turnover in whole mouse brain and in mouse cortex, cerebellum, brainstem and diencephalon [1, 2], as well as in rat hypothalamus and cortex [4], while others have reported no such increases in either whole rat brain or in several brain regions [3, 7]. However, when NE turnover was estimated by determining the rate of NE depletion after synthesis inhibition by alpha-methyl-paratyrosine, increases in NE turnover have been reported in the pons-medulla but not in other brain regions or in whole rat brain [6, 8, 9].

The effects of chronic administration of morphine on NE turnover in whole brain or brain regions are also contradictory. Whereas one group of investigators have observed tolerance to the morphine-induced increase of NE turnover in whole mouse brain [2, 10], other investigators have observed an increase in NE turnover in whole rat brain or in those brain regions that were examined after chronic morphine administration [4, 7].

Using the endogenous levels of the sulfate conjugate of 3-methoxy-4-hydroxyphenylglycol (MHPG-SO_4) the major metabolite of NE in rat brain [11] as an index of NE turnover, we have found that, in rats, acute administration of morphine produces a dose-related increase in the levels of MHPG-SO_4 in whole brain, and that tolerance develops to this effect of morphine after prolonged treatment [12]. We now report the differential effects of acute and chronic administration of morphine on MHPG-SO_4 levels in several regions of rat brain.

MATERIALS AND METHODS

Male Sprague–Dawley rats weighing 200–220 g were used throughout these experiments. In the acute experiments, rats were injected intraperitoneally with morphine sulfate (25 mg/kg) or isotonic saline (1 ml/kg) 2 hr prior to sacrifice. In the chronic experiments, animals received two injections of morphine sulfate per day commencing with a dose of 15 mg/kg each injection on the first day and increasing each injection by 15 mg/kg/day until a dose of 75 mg/kg each injection was attained on the fifth day. This dose was maintained for one additional day such that each rat received a total of 12 injections. Isotonic saline was administered to a control group following the same schedule of drug administration.

In one set of chronic experiments, animals were sacrificed 16 hr after the last maintenance dose of morphine or saline. In another set of experiments, animals chronically treated with morphine received a challenge dose of morphine sulfate (25 mg/kg) 16 hr after the last maintenance dose of morphine, and controls that had been chronically injected with isotonic saline were challenged with saline (1 ml/kg). Animals

* Dr. Roffman's current address is: Dr. Mark Roffman, Department of Pharmacology, CIBA-Geigy Corporation, Summit, NJ 07901.

Table 1. Effects of acute administration of morphine on the levels of MHPG-SO_4 in rat brain regions

	MHPG-SO_4 (pmoles/g brain tissue)		
	Control	Morphine	P
Cortex	383 ± 14	405 ± 20	N.S.
Corpus striatum	351 ± 29	368 ± 31	N.S.
Hypothalamus	620 ± 46	1008 ± 78	<0.01
Cerebellum	136 ± 14	200 ± 21	<0.01
Brainstem	494 ± 30	625 ± 29	<0.01
"Rest of brain"	570 ± 24	703 ± 61	<0.05

The experimental group was injected intraperitoneally with morphine sulfate (25 mg/kg) and the control group received isotonic saline (1 ml/kg). Animals were sacrificed by decapitation 2 hr after injections. Results are expressed as means ± standard errors of the means of 5–16 determinations.

were sacrificed by decapitation 2 hr after challenge injections.

Whole brains were removed, rinsed with cold saline and placed on iced-glass for dissection into hypothalamus, corpus striatum, cortex, cerebellum, brainstem (pons-medulla) and the remainder of the brain ("rest of brain") using a modification of the method of Glowinski and Iversen [13]. These tissues were weighed, frozen in dry ice-methanol and stored at −80° until assayed.

MHPG-SO_4 was estimated by the method of Meek and Neff [14] with minor modifications [12]. The tissues from 3 animals were pooled for assays of MHPG-SO_4 in each brain region. For technical purposes of the assay, only the right cortex was used in these experiments. Data from preliminary studies showed that there were no differences in MHPG-SO_4 levels between left and right cortices of saline or morphine-treated animals. Student's *t*-test was used to determine the statistical significance of differences between experimental and control groups.

RESULTS

The effects of a single injection of morphine (25 mg/kg) on MHPG-SO_4 levels in several brain regions are presented in Table 1. Acute administration of morphine significantly increased levels of MHPG-SO_4 in hypothalamus, cerebellum, brainstem and "rest of brain", but not in cortex or corpus striatum.

After chronic administration of morphine, following the schedule described in Methods, MHPG-SO_4 levels were examined 16 hr after the last dose of morphine. As shown in Table 2, the levels of MHPG-SO_4 were significantly decreased in the hypothalamus, cerebellum and "rest of brain". In the cortex and brainstem, the levels of MHPG-SO_4 were not significantly reduced (Table 2).

With chronic administration of morphine, tolerance appears to develop to the effects of morphine on NE turnover in cerebellum, brainstem and "rest of brain", since the challenge dose of morphine (25 mg/kg) did not produce significant changes in MHPG-SO_4 levels in these brain regions (Table 3). However, after chronic treatment the challenge dose of morphine did produce a significant increase in the levels of MHPG-SO_4 in the hypothalamus, suggesting that tolerance did not develop to the effects of morphine on NE turnover in hypothalamus under the conditions of these experiments (Table 3). Morphine (25 mg/kg) did not produce changes in MHPG-SO_4 levels in cortex of animals treated chronically with morphine (Table 3), just as this dose of morphine failed to alter MHPG-SO_4 levels in the cortex of naive animals (Table 1).

DISCUSSION

The results of this study indicate that acute administration of morphine does not effect NE turnover uniformly in all regions of rat brain. An acute injection of morphine increased MHPG-SO_4 levels in the hypothalamus, cerebellum, brainstem and "rest of brain", but not in cortex or corpus striatum. After

Table 2. MHPG-SO_4 Levels in rat brain regions following cessation of chronic morphine treatment

	MHPG-SO_4 (pmoles/g brain tissue)		
	Control	Morphine	P
Cortex	320 ± 10	308 ± 8	N.S.
Hypothalamus	606 ± 49	398 ± 21	<0.005
Cerebellum	117 ± 10	89 ± 6	<0.01
Brainstem	544 ± 17	508 ± 20	N.S.
"Rest of brain"	570 ± 26	464 ± 19	<0.005

The experimental group was injected intraperitoneally twice daily for six days with increasing doses of morphine sulfate as described in Methods. Control animals received saline (1 ml/kg) following the same schedule. Animals were sacrificed 16 hr after the last injection. Results are expressed as means ± standard errors of the means of 6 determinations.

Table 3. Effects of a challenge dose of morphine (25 mg/kg) on the levels of MHPG-SO_4 in rat brain regions after chronic treatment with morphine

	MHPG-SO_4 (pmoles/g brain tissue)		
	Control	Morphine	P
Cortex	355 ± 34	373 ± 27	N.S.
Hypothalamus	580 ± 49	747 ± 51	<0.01
Cerebellum	113 ± 13	90 ± 7	N.S.
Brainstem	558 ± 26	542 ± 14	N.S.
"Rest of brain"	544 ± 59	431 ± 60	N.S.

The experimental group was injected intraperitoneally twice daily for six days with increasing doses of morphine sulfate, as described in Methods. The control group received chronic injections of saline (1 ml/kg) according to the same schedule. Sixteen hr after the last of the chronic injections, experimental animals were administered a challenge dose of morphine (25 mg/kg), and control animals received a challenge dose of saline (1 ml/kg). Animals were sacrificed 2 hr after the challenge injections. Results are expressed as means ± standard errors of the means of 6–12 determinations.

the acute administration of morphine, Smith *et al.* [2] observed an increase in the synthesis of radioactively labelled NE from labelled tyrosine in various regions of mouse brain including hypothalamus, cortex, cerebellum, diencephalon and pons-medulla, but not in corpus striatum. It is possible that the discrepancy between our results and those of Smith with regard to cortex, may be related to species differences or to the doses of morphine employed. Using a procedure similar to that of Smith and colleagues [2], Johnson *et al.* [4] did observe an increase in NE turnover in rat cortex using a larger dose of morphine (60 mg/kg) than was employed in the present study.

Our studies using levels of endogenously-formed MHPG-SO_4 as an index of NE turnover, and studies of some other investigators who examined the synthesis of radioactively labelled NE from labelled tyrosine [2, 4], showed that morphine increased NE turnover in regions containing noradrenergic terminals (e.g., hypothalamus, cerebellum) as well as in regions containing noradrenergic cell bodies (e.g., brainstem). In contrast, several studies which utilized the rate of decline of endogenous NE after synthesis inhibition by alpha-methyl-paratyrosine as an index of NE turnover found morphine-induced changes only in pons-medulla, a region containing noradrenergic cell bodies [6, 9]. Thus, methodological differences may help to account for differences in the results of various studies of the effects of morphine on NE turnover in rat brain regions.

Following cessation of chronic treatment with morphine, the levels of MHPG-SO_4 were significantly reduced in the hypothalamus, cerebellum and "rest of brain" when animals were sacrificed 16 hr after the last dose. These data are consistent with the results of previous studies that reported a reduction in NE turnover in the whole brains of rats [12] and mice [2] after the cessation of chronic administration of morphine.

Under the experimental conditions of this study, after long-term administration of morphine, tolerance appeared to develop to its effects on NE turnover in cerebellum, brainstem and "rest of brain", but not in the hypothalamus where the challenge dose of morphine significantly increased MHPG-SO_4 levels in rats chronically-treated with the drug (Table 3). That the hypothalamus did not show tolerance to the effects of morphine cannot be ascribed to higher "baseline" levels of MHPG-SO_4 in the hypothalamus after chronic morphine, since the "baseline" levels of MHPG-SO_4 in the hypothalamus were significantly decreased prior to the challenge dose of morphine (Table 2).

Recent studies have shown that tolerance does not appear to develop to the effects of morphine on the threshold and rates of responding for self-stimulation in the lateral hypothalamus [15, 16], and in the present study, tolerance did not develop to the effects of morphine on NE turnover in the hypothalamus. Although other neuronal systems clearly may be involved in the effects of morphine on self-stimulation [17], the present findings are compatible with the hypothesis that the effects of morphine on hypothalamic self-stimulation may be mediated, at least in part, by noradrenergic neuronal systems.

Acknowledgements—This work was supported in part by Grants No. DA 00257 and No. DA 01460 from the National Institute on Drug Abuse. Hoffmann-La Roche Inc. of Nutley, New Jersey generously provided the MHPG-SO_4 used in these studies. The authors wish to thank Mr. Mitchel Kling for his technical assistance.

REFERENCES

1. C. B. Smith, J. E. Villarreal, J. H. Bednarczyk and M. I. Sheldon, *Science, N.Y.* **170**, 1106 (1970).
2. C. B. Smith, M. I. Sheldon, J. H. Bednarczyk and J. E. Villareal, *J. Pharmac. exp. Ther.* **180**, 547 (1972).
3. E. Costa, A. Carnezi, A. Guidotti and A. Revuelta, in *Frontiers in Catecholamine Research* (Eds E. Usdin and S. Snyder) p. 1003. Pergamon Press, New York (1973).
4. J. C. Johnson, M. Ratner, G. Gold and D. Clouet, *Res. Commun. Chem. Path. Pharmac.* **9**, 41 (1974).
5. S. K. Puri, C. Reddy and H. Lal, *Res. Commun. Chem. Path. Pharmac.* **5**, 389 (1973).
6. M. F. Sugrue, *Br. J. Pharmac.* **52**, 159 (1974).
7. D. H. Clouet and M. Ratner, *Science N.Y.* **168**, 854 (1970).
8. L.-M. Gunne, J. Jonsson and K. Fuxe, *Eur. J. Pharmac.* **5**, 338 (1969).
9. G. Paalzow and L. Paalzow, *Psychopharmacologia* **45**, 9 (1975).

10. S. J. Rosenman and C. B. Smith, *Nature, Lond.* **240**, 153 (1972).
11. S. M. Schanberg, J. J. Schildkraut, G. R. Breese and I. J. Kopin, *Biochem. Pharmac.* **17**, 247 (1968).
12. M. Roffman, T. G. Reigle, P. J. Orsulak and J. J. Schildkraut, *Res. Commun. Chem. Path. Pharmac.* **10**, 403 (1975).
13. J. Glowinski and L. Iversen, *J. Neurochem.* **13**, 655 (1966).
14. J. L. Meek and N. H. Neff, *J. Pharmac. exp. Ther.* **184**, 570 (1973).
15. A. Pert in *Problems of Drug Dependence: 1975*, pp. 666–677. National Research Council, National Academy of Science, Washington, DC (1975).
16. R. Esposito and C. Kornetsky, *Science*, **195**, 189 (1977).
17. D. German and D. Bowden, *Brain Res.* **73**, 381 (1974).