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Single-dose tolerance to the effects of morphine on brain 3-methoxy-4-hydroxyphenylethylene glycol sulfate*

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Measurements of the concentration of 3-methoxy-4-hydroxyphenylethylene glycol sulfate (MOPEG-SO_4), the major rat brain metabolite of norepinephrine [1], have been used in several recent studies to obtain an index of the effects of various treatments on brain norepinephrine turnover [2–4]. In agreement with previous studies demonstrating an increased synthesis of brain norepinephrine from radiolabeled tyrosine [5], the acute administration of morphine has been shown to produce dose-dependent increases in MOPEG-SO_4 in whole rat brain [6] and several of its parts [7]. Recent evidence from our laboratory indicates that this observed increase in brain norepinephrine turnover is a specific opiate receptor effect [8]. Thus, morphine and related opiate agonists produce dose-dependent increases in rat brain MOPEG-SO_4 that exhibit characteristic potency differences, stereospecificity, and naloxone antagonism, and that do not occur as a result of interference with the transport of this major metabolite from brain [8].

An additional criterion for specific opiate action is the development of tolerance, and the effect of morphine on brain MOPEG-SO_4 has been shown to be attenuated following chronic administration [6]. However, significant tolerance to the analgesic effect of morphine has been demonstrated following the administration of a single dose of the opiate [9], and single-dose morphine tolerance has also been exhibited by the depletion of rat brain calcium [10]. The requirement for newly synthesized protein in tolerance development has been implicated in both of these instances by demonstrations of tolerance prevention after prior administration of protein synthesis inhibitors [10, 11]. The present study was designed to further characterize the relationships between increased brain norepinephrine turnover, opiate action, and tolerance development by evaluating the ability of morphine to induce single-dose tolerance to its effect on brain MOPEG-SO_4 and the ability of cycloheximide, an inhibitor of protein synthesis, to antagonize the development of single-dose tolerance to this effect.

Male Sprague–Dawley rats (Holtzman, 170–230 g) were injected i.p. with either isotonic sodium chloride or a 10 mg/kg dose of morphine sulfate (Mallinckrodt, Inc., St. Louis, MO). Twenty-four hours after this initial injection, one of several doses of morphine was administered, and the animals were decapitated 1 hr following the second injection at the time of the peak effect on brain MOPEG-SO_4 [8]. Whole brain MOPEG-SO_4 concentrations were determined by the fluorometric method of Meek and Neff [12]. In experiments designed to evaluate the antagonism of tolerance, a 1 or 2 mg/kg i.p. dose of cycloheximide (Aldrich Chemical Co., Milwaukee, WI) was administered 1 hr prior to the initial 10 mg/kg injection of morphine [10], and the subsequent schedule was repeated exactly as described above. The effect of cycloheximide (2 mg/kg) on the increase in brain MOPEG-SO_4 produced by single injections of morphine was determined by killing groups of cycloheximide (1 hr and 24 hr) pretreated animals 1 hr after the administration of morphine (10 mg/kg). Brain MOPEG-SO_4 levels were expressed as picomoles per gram of brain (wet weight), and appropriate statistical comparisons were made with a one-way analysis of variance and Student's t-test ($\alpha = 0.05$).

The degree of protein synthesis inhibition produced by the intraperitoneal administration of these doses of cycloheximide in rats was determined by measuring the incorporation of L-[U-^{14}C]valine (ICN Pharmaceuticals, Irvine, CA, 250 Ci/mole) into brain protein according to the procedure of Sperk *et al.* [13]. Rats were injected with saline or cycloheximide (1 or 2 mg/kg, i.p.) 1 hr before intraperitoneal administration of [^{14}C]valine and killed 1 hr after. The [^{14}C]valine was diluted with a saturated solution of cold valine (50 mg/ml) so that the final concentration of the label was 2.5 μCi/ml and each rat was given 5 μCi/100 g of body weight. Brains were homogenized in 10 ml of 10% (w/v) trichloroacetic acid (TCA). Following lipid extraction, the pellets were dried, weighed, dissolved by heating in 1 N NaOH, neutralized with glacial acetic acid, and added to 15 ml of commercial counting solution (Scintiverse, Fisher Scientific, Fair Lawn, NJ). Radioactivity was

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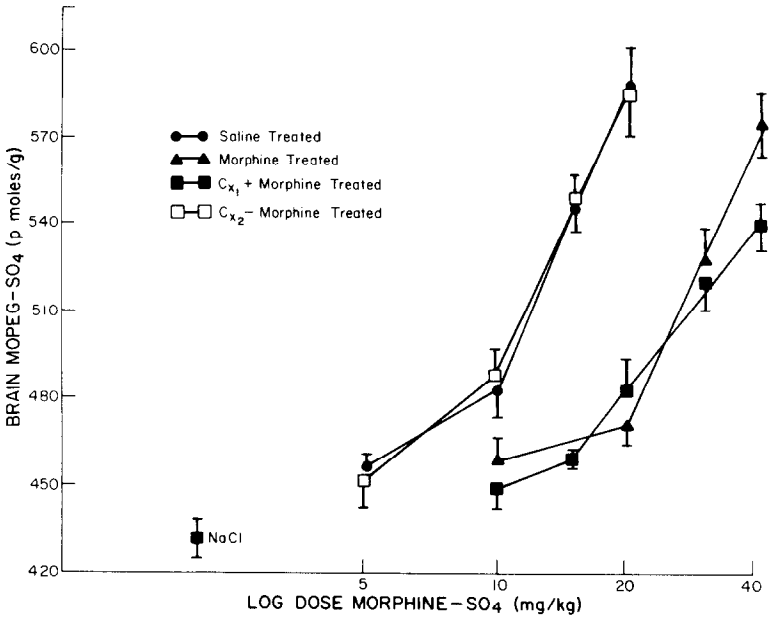

Fig. 1. Log dose–response curves of the effect of morphine on brain MOPEG-SO_4 in rats treated 24 hr previously with saline, morphine (10 mg/kg i.p.), or cycloheximide (1 and 2 mg/kg, i.p., 1 hr; Cx_1 and Cx_2) plus morphine (10 mg/kg i.p.). Animals were killed 1 hr after the indicated doses of morphine. NaCl (■) = saline control (432 ± 7 pmoles/g) measured 1 hr after two saline injections given 24 hr apart. Vertical bracketed lines indicate S.E.M. N = eight to twelve animals for each dose of morphine examined.

counted in a Beckman LS-100C scintillation counter utilizing quench correction by external standard and channels ratio, and [^{14}C]valine in TCA-precipitable protein was expressed as disintegrations per minute per milligram pellet.

In agreement with previous findings [8], morphine doses between 5 and 20 mg/kg produced significant, dose-dependent increases in the concentration of brain MOPEG-SO_4 in 24-hr saline-pretreated controls (Fig. 1, ●——●). Dose-dependent increases in brain MOPEG-SO_4 were also produced in animals treated with morphine (10 mg/kg) 24 hr prior to the administration of opiate challenge doses. Tolerance, to this effect, however, was evidenced by more than a 2-fold shift of the dose–response curve to the right (Fig. 1). A shift of the dose–response curve to the right was also obtained in animals treated with a 1 mg/kg dose of cycloheximide 1 hr prior to the initial injection of morphine. But, as evidenced by the generation of a dose–response curve superimposed on that obtained in 24-hr saline-pretreated controls, a 2 mg/kg dose of cycloheximide 1 hr prior to the initial administration of morphine (10 mg/kg) totally prevented single-dose tolerance to the effect of morphine on brain MOPEG-SO_4 (Fig. 1). Brain concentrations of MOPEG-SO_4 were not significantly different from control 24 hr after a single 10 mg/kg dose of morphine (424 ± 11 vs 423 ± 5 pmoles/g).

Additional experiments demonstrated that cycloheximide pretreatment (2 mg/kg) given 1 or 24 hr prior to morphine did not alter the increase in brain MOPEG-SO_4 produced 1 hr after a single 10 mg/kg injection of the opiate (NaCl = 433 ± 4, M_{10} = 483 ± 9, Cx_2 + M_{10} = 483 ± 6 pmoles/g for 1 hr, Cx_2:NaCl = 438 ± 7, M_{10} = 467 ± 6, Cx_2 + M_{10} = 477 ± 5 pmoles/g for 24 hr Cx_2). Further, cycloheximide alone (2 mg/kg) had no significant effect on brain MOPEG-SO_4 concentration at times corresponding either to the scheduled injections of morphine or the subsequent MOPEG-SO_4 analysis in the single-dose tolerance experiments described above, i.e. 1 hr (425 ± 10 pmoles/g) or 24–26 hr (429 ± 7 to 441 ± 6 pmoles/g) after cycloheximide administration.

The effects of cycloheximide (1 and 2 mg/kg, i.p.) on the incorporation of [^{14}C]valine into brain protein are shown in Table 1. These data indicate that, although brain protein synthesis was inhibited more than 50 per cent 1–2 hr after both doses of cycloheximide, 2 mg/kg of this agent produced a significantly greater inhibition than 1 mg/kg ($P < 0.02$).

The results of this study illustrate the development of single-dose tolerance to the effect of morphine on brain MOPEG-SO_4 and the prevention of this tolerance by prior administration of sufficient doses of cycloheximide. These findings are comparable to those obtained from evaluations of the analgesic, and other, actions of morphine [11, 10] and, together with previous evidence [8], greatly strengthen the concept that the production of an increase in brain norepinephrine turnover is a specific component of the pharmacological actions of narcotic analgesics.

The requirement for brain protein synthesis has been incorporated into several theories concerning the mechanism of opiate tolerance development [11], and the prevention of tolerance by cycloheximide suggests that single-dose tolerance to both analgesia and enhanced norepinephrine turnover requires an activated production of protein. It has also been suggested that cycloheximide is effective in inhibiting tolerance development only when protein synthesis is maximally suppressed [14]. Since a 63 per cent inhibition of brain protein synthesis was apparently sufficient to prevent single-dose tolerance to the increase in brain MOPEG-SO_4 produced by morphine in the present study but a 52 per cent inhibition apparently was not (Table 1), the present findings suggest that levels of protein critical to the initiation of single-dose opiate tolerance are sufficiently suppressed when 50–60 per cent of total brain protein synthesis is inhibited.

Cycloheximide (2 mg/kg) administered 1 or 24 hr prior to morphine had no effect on the increase in brain MOPEG-SO_4 produced by a single injection of the opiate. Thus, this

Table 1. Effects of cycloheximide (Cx) on the incorporation of [^{14}C]valine into brain protein*

Treatment	[^{14}C]valine (dpm/mg pellet)	% Inhibition
Saline	8.39 ± 0.20 (7)	
Cx (1 mg/kg, i.p.)	3.99 ± 0.12† (7)	52.4 ± 1.4†
Cx (2 mg/kg, i.p.)	3.09 ± 0.25†,‡ (8)	63.2 ± 3.0†,‡

* Rats were injected with saline or cycloheximide 1 hr prior to the i.p. administration of [^{14}C]valine (5 μCi/100 g) and killed 1 hr thereafter. Brains were removed and assayed for [^{14}C]valine in TCA-precipitable protein by the method of Sperk *et al.* [13]. Results are means ± S.E.M. of the number of animals indicated in parentheses.

† $P < 0.01$ vs saline.

‡ $P < 0.02$ vs Cx_1.

protein synthesis inhibitor did not exert a delayed effect on the initial morphine response, and increased protein synthesis was not required for the increase in brain norepinephrine turnover that was observed. The enhancement of brain norepinephrine turnover may, therefore, occur prior to any activation of protein synthesis that these drugs produce.

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