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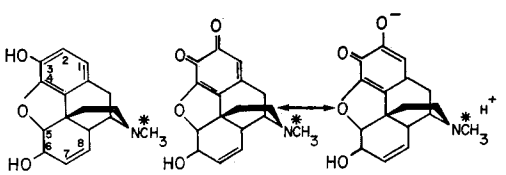
Some physicochemical and pharmacological properties of morphine-2,3-quinone, the morphine metabolite in the rat brain

Evidence for the formation *in vitro* and *in vivo* of a 2,3-catechol type of metabolite by aromatic hydroxylation of morphine in rat brain has been presented earlier (Misra, Mitchell & Woods, 1971; Misra, Vadlamani & others, 1973). The chromatographic properties of this metabolite were similar to the zwitterionic morphine-2,3-quinone. This communication describes some physicochemical, pharmacological properties and binding characteristics *in vitro* of [^{14}C]morphine-2,3-quinone with some biological macromolecules.

[^{14}C]Morphine-2,3-quinone was prepared from morphine-*N*-Me[^{14}C] as previously described (Misra & others, 1973). The partition coefficients of water-soluble [^{14}C]-morphine-2,3-quinone and [^{14}C]morphine in 1-octanol–phosphate buffer pH 7·4 at



[^{14}C]Morphine [^{14}C]Morphine-2,3-quinone

*Position of label

ambient temperature as measured by Misra, Pontani & Mulé (1974) were 0·006 and 1·0, respectively. Amberlite XAD-2 resin adsorbed only 47% of the quinone compared with the quantitative adsorption of morphine from aqueous solutions and the adsorbed compound could be eluted with methanol.

Groups of 10 male Wistar rats (100–150 g) were lightly anaesthetized with ether and injected intracisternally with morphine or morphine-2,3-quinone in 10 μl using a Hamilton syringe and 27 gauge $\times$ $\frac{3}{8}$ inch needle. The pH of the two injection solutions was adjusted to 6·9–7·0 with 0·1 N NaOH. Analgesia was tested 0·5, 1, 2 and 4 h post-injection on a hot plate (55°) with a cut-off time of 30 s. A 10 mg kg^{-1} intravenous dose (tail vein) of morphine-2,3-quinone did not produce any analgesia or narcosis, sedation or respiratory depression in the rat. Repeated 10 mg kg^{-1} intravenous injections of morphine-2,3-quinone twice daily for 4 days also did not affect the onset of analgesia produced by a subsequent injection of 10 mg kg^{-1} (s.c.) dose of morphine. Intracisternal administration of a 0·5 mg kg^{-1} dose of morphine-2-3-quinone, however, produced rapid breathing and heart rate, disorientation, jumping and clonic-tonic convulsions in all animals. Doses of morphine-2,3-quinone (1 mg kg^{-1}) administered intracisternally caused death by convulsions within 15–20 min in all animals, while a similar dose of morphine only produced analgesia, sedation and respiratory depression in all animals. Mixtures of morphine (0·5 mg kg^{-1}) and morphine-2,3-quinone (0·5 mg kg^{-1}) administered intracisternally also caused convulsions and death in some animals, while doses less than 0·5 mg kg^{-1} of morphine-2,3-quinone alone had no observable pharmacological effect. The highly polar character of morphine-2,3-quinone prevents its penetration through the blood-brain barrier on systemic administration.

Studies on binding of [^{14}C]morphine and [^{14}C]morphine-2,3-quinone with human γ-globulin (7S fraction) and fraction II (Table 1) by the method of Misra & others (1974) were undertaken, in view of the observations that an antibodylike, morphine-binding immunoglobulin formed in rabbit serum on repeated daily administrations

Table 1. *Binding*[a] *of* [N-*methyl*-^{14}C]*morphine, and* [N-*methyl*-^{14}C]*morphine*-2,3-*quinone with human* γ-*globulin* (7S)[b] *fraction and* γ-*globulin* (*fraction II*) in vitro.

Drug and concn range used (μg ml^{-1})	Concn (%)		Binding (%)
	Human γ-globulin(7S)	γ-globulin (Fraction II)	
[^{14}C]Morphine (1–100)	0·75—1·5	—	3·5 ± 0·7
[^{14}C]Morphine-2,3-quinone (2–200)	0·75	—	14·6 ± 1·3
[^{14}C]Morphine-2,3-quinone (2–200)	1·5	—	13·9 ± 2·1
[^{14}C]Morphine-2,3-quinone (20) + nonlabelled morphine (1–1000)	0·75	—	13·9 ± 1·3
[^{14}C]Morphine-2,3-quinone (200) + nonlabelled morphine (10–1000)	0·75	—	16·0 ± 1·9
[^{14}C]Morphine-2,3-quinone (10) + nonlabelled naloxone (1000)	1·5	—	11·3 ± 1·7
[^{14}C]Morphine-2,3-quinone (20–200), pH 6·8	0·75	—	Same as that at pH 7·4
[^{14}C]Morphine-2,3-quinone (20–200), pH 8·0	0·75	—	0
[^{14}C]Morphine-2,3-quinone (1–100)	—	0·75	6·5 ± 1·6
[^{14}C]Morphine (1–100)	—	0·75	6·2*
[^{14}C]Morphine-2,3-quinone + nonlabelled morphine	—	0·75	0

(a) Binding unless otherwise mentioned, was studied using human grade A, γ-globulin (7S) or γ-globulin (fraction II) in M/15 phosphate buffer pH 7·4 at 37°. Data represent the mean ±s.e., * represents the mean of 2 determinations.

(b) Human γ-globulin (7S) fraction, A grade, was obtained from Calbiochem, San Diego, California; γ-globulin (fraction II) from Nutritional Biochemicals Corporation, Cleveland, Ohio.

of morphine (Ringle & Herndon, 1972), and significant binding of morphine by serum globulins (Ryan, Parker & Williams, 1972) and significant increase in serum immunoglobulins (Weksler, Cherubin & others, 1973) occurred in heroin addicts. Significantly greater binding *in vitro* of [^{14}C]quinone (14·6 ± 1·3%) compared with [^{14}C]morphine (3·5 ± 0·7%) was observed with human γ-globulin (7S fraction). Similar binding was also observed at pH 6·8 but not at pH 8·0 and this binding was not inhibited by non-labelled morphine or naloxone. Prolonged dialysis was required to dissociate the quinone-protein complex compared with the rapid dissociation of the morphine-protein complex. Binding of [^{14}C]morphine quinone with γ-globulin (fraction II) (6·5 ± 1·6%) was similar to that with morphine and was completely inhibited by non-labelled morphine. The values on binding of morphine with γ-globulin (fraction II) are in the range reported by Olsen (1973a,b). Binding of [^{14}C]quinone in the concentration range (1–100 μg ml^{-1}) with rat brain homogenates (10% in M/15 phosphate buffer, pH 7·4) occurred to the extent of 15·3 ± 1·6% (mean ± s.e.), only in presence of reduced glutathione (20 mg).

Morphine possesses both depressant and stimulant properties (Seevers & Woods, 1953; Seevers & Deneau, 1962). The present study shows that morphine-2,3-quinone has potent stimulant properties on intracisternal administration and in equal doses (0·5 mg kg^{-1}) could override the depressant effects of morphine injected by the same route. The greater affinity and binding of quinone with protein compared to morphine would account for its persistence in the rat brain (Misra & others, 1971). Repeated administration of morphine could conceivably lead to a gradual increase in the content of quinone in the cns. Although it is not possible at the present time to postulate any causal relation between the stimulant properties of the quinone and distinctly complex manifestations of abstinence signs on withdrawal of morphine, three distinct possibilities must not be overlooked: (*a*) that the depressant actions of morphine could mask the stimulant actions of quinone formed *in situ* in the cns during the course of morphine administration, (*b*) a disruption of the balance of depressant and stimulant effects upon termination of morphine could result in unmasking the stimulant properties of quinone, and (*c*) such a closely related metabolite formed at or near the receptor for morphine could compete for that site and lead to its blockade.

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