Clearance and analgesic activity of the quaternized opiate, *N*-methylmorphine [6-^{3}H] administered intracisternally to the rat

A. L. MISRA*, N. L. VADLAMANI, R. B. PONTANI, *New York State Office of Drug Abuse Services Research Laboratory, 80 Hanson Place, Brooklyn, New York, 11217, U.S.A.*

Highly polar quaternized opiates e.g. *N*-methylmorphine, which are not able to cross the blood-brain barrier, have been reported to produce analgesia and hypothermia when injected intraventricularly or into the periventricular grey matter of the rat (Foster, Jenden & Lomax 1967; Herz & Teschemacher, 1971). Opiate-like effects for these compounds have also been demonstrated in the guinea-pig ileum preparation (Kosterlitz, Lord & Watt 1972). Quaternized opiate antagonists likewise, were reported to possess antagonist properties (Zeile & Freter, 1961). Typical stereospecific, naloxone-reversible effects in the guinea-pig ileum longitudinal muscle preparation have recently been reported for quaternized levorphanol (Opheim & Cox, 1976). These studies have led to the conclusion that such compounds must interact with opiate receptors located on the external surface of the neuronal membrane.

We report on the preparation, clearance and analgesic activity of *N*-methylmorphine [6-^{3}H] administered intracisternally to the rat and show that the analgesia is due to the quaternary compound and not to an *in vivo* conversion to the tertiary amine.

N-Methylmorphine iodide was prepared as follows. To a solution of morphine base (285 mg) in hot absolute ethanol (10 ml) in a 50 ml pear-shaped flask, methyl iodide (0·2 ml) was added and the contents heated under reflux for 2 h in a silicone oil bath (105°). The crystalline mass formed was filtered and recrystallized twice from absolute methanol (yield 280 mg), m.p. 263–265° (decomp.). Found C, 50·44; H, 5·18%: Calc. for $C_{18}H_{22}INO_3$, C, 50·58: H, 5·15%. On t.l.c. (Silica gel, Gelman Instrument Co., Ann Arbor, Mich.) in solvent system (a) ethyl acetate–methanol–conc. ammonia (17:2:1 v/v), the compound gave R_F values of 1·0 and 0·0 for morphine and *N*-methyl morphine iodide respectively and in system (b) n-butanol–acetic acid–water (35:3:10 v/v), it gave R_F values of 0·92 and 0·68 for morphine and *N*-methyl morphine iodide respectively.

[6-^{3}H]Morphine base (28·5 mg) in absolute ethanol (2 ml) in a 5 ml pear-shaped flask reacted with methyl iodide (25 μl) under the above conditions gave *N*-methyl morphine [6-^{3}H] iodide (28 mg), specific activity 5·1 μCi mg^{-1}, radiochemical purity 99% checked by t.l.c. in solvent systems a and b.

Male Wistar rats (170–200 g) were lightly anaesthetized with ether and injected intracisternally (i.c.) with a 0·5 mg kg^{-1} *N*-methylmorphine[6-^{3}H] iodide in 0·9% saline. The total volume of injection solution was 8–10 μl and the concentration 10 mg ml^{-1}. At various times after injection, the blood was removed by cardiac puncture and immediately centrifuged to remove the plasma. Whole brains were rinsed with cold 0·9% saline, wiped, weighed, transferred to a counting vial, NCS tissue solubilizer (Amersham-Searle Corp., Arlington Heights, Illinois, U.S.A.) (5·5 ml) was added and the tubes, lightly capped, were left on a Fisher-Slide Warmer at 45° for 4 days. Radioactivity in dissolved tissue samples was counted, after addition of toluene-phosphor (10 ml), in a liquid scintillation counter and counts were corrected for quenching by using [^{3}H] toluene as internal standard. Known aliquots (0·5 ml) of diluted plasma were mixed with NCS (1·5 ml), warmed and the radioactivity counted as before. Appropriate controls for brain and plasma from rats injected with 0·9% saline (i.c.) were concurrently carried out to serve as blanks for obtaining absolute counts min^{-1} g^{-1} of tissue or ml^{-1} of plasma.

The comparative analgesic effects of morphine and *N*-methylmorphine iodide were assessed in male Wistar rats (150–200 g), which were divided into groups of 5, lightly anaesthetized with ether and injected (i.c.) with 0·9% saline (control) or 0·5 mg kg^{-1} morphine ($\equiv$ to a 10–20 mg kg^{-1} dose, s.c. in analgesic activity) or the quaternary compound in 8–10 μl (injection solution 10 mg ml^{-1}). The mean latencies of pain response (s) in these animals were determined on a hot plate (55°) at different times after injection. Licking of one paw or intensive jerking with lifting off or jumping of hind legs were the criteria. The test was stopped if the response latency exceeded 30 s.

Table 1. *Decay of radioactivity* of* N-*methylmorphine* [6-^{3}*H*] *from brain and plasma of rats injected intracisternally with* 0·5 *mg kg*$^{-1}$ *dose of the labelled compound.*

	0·5 h	1 h	2 h	4 h	6 h	App. $t_{\frac{1}{2}}$† (h)
Brain	37130	19660	9610	4730	2370	1·48
Plasma	1010	615	285	105	85	1·54
Plasma: Brain	0·027	0·031	0·030	0·022	0·036	

* The values represent mean absolute counts min^{-1} g^{-1} or ml^{-1} fluid from 2 animals at each time. The deviation of values from the mean in brain at 1, 2 h was 13 to 15%, that at other times 0·5 to 4%. The deviation of values from the mean in plasma ranged between 5 to 20%.

† Apparent $t_{\frac{1}{2}}$ were calculated from semi-logarithmic regression plots of values in brain and plasma. Comparison of the slopes of regression lines by *t*-test showed that $t_{\frac{1}{2}}$ values in brain and plasma were not significantly different.

* Correspondence.

For t.l.c., plasma samples from rats killed at different times (0·5–24 h) were pooled, diluted with water and repeatedly passed through a column of Amberlite XAD-2 (1 × 10 cm). The column was washed with a column length of water and drug eluted with absolute methanol (250 ml). The eluate was evaporated to dryness under vacuum and the residue chromatographed using solvent systems a and b.

The clearance of *N*-methylmorphine [6-^{3}H] from rat brain and plasma after a 0·5 mg kg^{-1} dose by intracisternal injection appears in Table 1. The $t_{\frac{1}{2}}$ values of decay in brain and plasma were approximately 1·48 and 1·54 h respectively. The percentage of dose present in brain 24 h after injection was approximately 0·6 and barely detectable in plasma. Although the measurement of radioactivity in whole brains may not directly reflect the actual concentrations of drug in the csf, the rate of efflux from these may still occur in a parallel fashion.

Similar values of plasma-brain ratios between 15 min and 6 h indicated a constant rate of efflux of drug from the csf into the plasma. Previous work (Davson & Bradbury, 1965; Oldendorf, 1974) has shown that the csf acts as a 'sink' for the diffusion of drugs from the extracellular fluid of the cns into the venous blood.

The chromatography of pooled plasma samples (0·5–24 h) did not reveal any biotransformation of *N*-methylmorphine to morphine indicating that the cationic protonated form of the opiate (quaternary compound) is the active species responsible for analgesia.

The comparative analgesic activities of morphine and *N*-methylmorphine after a dose of 0·5 mg kg^{-1} (i.c.) as determined on the hot plate appear in Fig. 1. The quaternary compound had substantially lower analgesic potency (0·1 and 0·25 mg kg^{-1}, i.c. had little analgesic action) and a shorter duration of action compared with morphine and produced morphine-like sedation after a brief period of initial excitation. The time course of efflux of *N*-methylmorphine from the cns into plasma correlated with its pharmacological activity. The substantially lower analgesic activity and duration of analgesia of *N*-methylmorphine could possibly arise from its low receptor affinity due to steric hindrance or reduced hydrogen bonding potential (Herz & Teschemacher, 1971; Opheim & Cox, 1976). Previous work by Kosterlitz & others (1972) has shown that *N*-methylmorphine had only 2·5% of the potency of morphine in guinea-pig ileum. The ID50s, the concentrations causing 50% inhibition of contraction of the ileum longitudinal muscle, for morphine and *N*-methylmorphine were 68, 2430 nM respectively and Ke, the reciprocal of affinity constant 88, 1823 nM respectively. *N*-Methylmorphine has recently been shown to depress the stereospecific binding of [^{3}H] naloxone (1nM) to mouse brain membranes with an ID50 of 8 nM (Smith, 1977). Our results are in accord with earlier observations of Kosterlitz & others (1972) and Opheim & Cox (1976) on *N*-methyllevorphanol. Foster & others (1967) however, reported equipotent analgesic activity for morphine and *N*-methyl morphine after injections into the periventricular grey matter of the rat. Differences in rates of efflux of quaternary opiate from the cns by intracisternal and intracerebral injections may possibly account for the reported differential biological potencies.

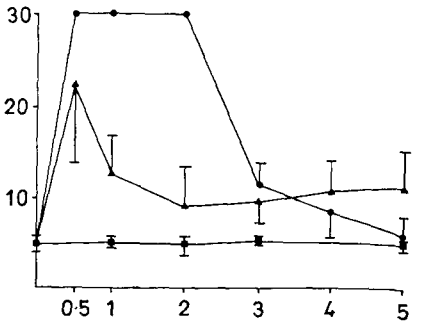

FIG. 1. Mean response latency (s) as assessed on the hot plate of rats (n=5) injected intracisternally with 0·5 mg kg^{-1} dose of morphine (●), *N*-methylmorphine (▲) or 0·9% saline (■). Latency (s) given as mean with s.d. on the ordinate and times after injection (h) on the abscissa. For saline controls, zero time denotes values obtained approximately 5 min after injection, when the effects of light ether anaesthesia had worn off. Area under the time-response curve (0–3 h) for morphine was significantly greater ($P < 0·05$) than that for *N*-methylmorphine using the paired *t*-test. The response latency to morphine reached the permissible maximum from 0·5–2 h.

The authors thank Dr S. J. Mulé for his interest and support. The technical assistance of Mr Milton Ratner and Mr George Casella is gratefully acknowledged.

August 18, 1977

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