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ASSESSMENT OF THE OPIATE PROPERTIES OF TWO CONSTITUENTS OF A TOXIC ILLICIT DRUG MIXTURE

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SUMMARY

The intravenous use of an illicit synthetic drug preparation has caused permanent parkinsonism in a number of addicts. Chemical analysis has revealed the ingredients to be two related compounds 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 1-methyl-4-phenyl-4-propionoxy-piperidine (MPPP). The opiate properties of these two compounds have been assessed using in vitro receptor binding techniques as well as behavioral tests indicative of opiate action, including analgesia, catatonia, respiratory depression and the loss of righting and corneal reflexes. All opiate activity was found to reside with MPPP, which proved to be a potent μ-type agonist. It is concluded that the opiate properties of MPPP alone explain repeated abuse of MPTP/MPPP mixtures by heroin addicts.

Key words: 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine — 1-Methyl-4-phenyl-4-propionoxypiperidine — Opiates

The intravenous use of an illicit synthetic drug preparation has been found to cause a permanent parkinsonism in humans [1,2]. Chemical analyses of powders obtained from drug abusers have shown the active constituents to consist of two related compounds: MPTP and MPPP, in ratios varying from 10:1 (MPTP/MPPP) to 1:100 [2]. These preparations produced a potent opiate 'high' as evidenced by verbal reports and by the repeated use of the compound over several consecutive days [12,]. The toxicity of this mixture has subsequently been found to be due to MPTP [3]. However, the opiate

Abbreviations: DADL, 2-D-Ala-5-D-Leu-enkephalin; DAGO; Tyr-D-Ala-Gly-MePhe-$NHCH_2CH_2OH$; MPPP, 1-methyl-4-phenyl-4-propionoxypiperidine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

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properties of these compounds, which presumably prompt repeated abuse, have not been characterized. While MPPP has been reported to have analgesic activity [4], the criteria of opiate receptor binding activity and naloxone reversability were not tested. This fact, coupled with the prevalence of MPTP in the drug mixture prompted us to examine in vitro and in vivo the opiate properties of both MPPP and MPTP.

METHODS

In vitro receptor binding

Adult male albino rats (Osborne-Mendel, NIH, 400—500 g) were decapitated and their brains rapidly removed. P2 fractions of whole brain were prepared by homogenization in 20 vol. of cold 0.32 M sucrose followed by low speed centrifugation (1000 × *g*, 10 min). The resulting supernatants were decanted and centrifuged at high speed (17 500 × *g*, 40 min) to obtain pellets (P2), which were frozen at − 70°C until needed.

Tritiated naloxone (48.6 Ci/mmol, New England Nuclear), μ-type agonist [5] DAGO (Tyr-D-Ala-Gly-MePhe-$NHCH_2CH_2OH$, 30 Ci/mmol, Amersham) and DADL (2-D-Ala-5-D-Leu-enkephalin, 32 Ci/mmol, New England Nuclear), a delta-selective agonist [6], were used as labeled ligands. The final concentration of all labeled ligands was 1 nM. The frozen P2 fractions were taken to a final concentration of 1 : 150 (original wet wt./vol.) in Tris buffer, 0.05 M (pH 7.4). Fifty microliter aliquots of labeled ligand and 50 μl of the competing ligand were added to disposable glass culture tubes (12 × 75 mm), followed by 0.9 ml of the tissue preparation. Incubation continued for 30 min at room temperature and was then terminated by rapid filtration under negative pressure through glass fiber filters (Whatman GF/B) followed by rapid washing with cold buffer (2 × 5 ml). Filters were placed in glass scintillation vials, 10 ml of Aquasol (New England Nuclear) was added and the radioactivity was counted 24 h later. All concentrations of competing ligand were tested in triplicate. The results are plotted as the log of the competing ligand concentration vs. dpm.

To assess the antagonist binding potential of MPPP and MPTP, these competing ligands were incubated with labeled naloxone for 1 h at 0°C in a Krebs-Tris buffer [7]. This high Na, low temperature incubation has been shown to favor pure or partial opiate antagonist binding [8].

Behavioral experiments

A total of 194 adult male rats (Osborne-Mendel, NIH, 400—500 g) were used in these studies. The animals were housed in pairs in clear plastic cages and given food and water ad lib. Solutions of all test compounds were made up in saline and injected subcutaneously over the scapulae in a volume of 1 ml/kg. To avoid complications due to drug tolerance, each rat was used in a single experiment only. Test compounds used were the hydrochloride salts of MPPP [1], naloxone (Endo Laboratories), meperidine (Demerol,

Winthrop) and alphaprodine (Nisentil, Roche), and morphine sulfate (Merck and Co.). MPTP (Aldrich) was converted to the hydrochloride by bubbling HCl gas through a 10% solution in dry hexane and recrystallizing from ethyl acetate and methanol. All dosages are expressed as μM/kg. All test groups consisted of 6—12 animals. Statistical comparisons were made between groups using Student's *t*-test.

The degree of analgesia was measured using the tail flick test [9]. Briefly, rats were restrained in clear plastic tubes to which they had been previously habituated. Their tails were exposed to a radiant heat source (the focused beam of a 100-W light bulb), and the latency from stimulus onset to a reflex flection was measured. The bulb voltage was adjusted so that the average baseline latency was about 4 s. To prevent tissue damage, the bulb was turned off automatically after 8 s if no reflex had occurred. The test procedure consisted of: (1) recording a baseline tail flick latency, (2) injecting the test solutions and (3) measuring the tail flick latency every 10 min for varying amounts of time. If analgesia was observed, its susceptibility to naloxone reversal was tested.

In order to compare the analgesic potency of the test compounds with standard analgesics, dose response curves were generated. The test compounds were compared in potency to the structurally-related compounds alphaprodine and meperidine as well as to morphine. The time of peak analgesic effect was determined for each compound, and the analgesia measured at this time for each drug was used to generate the curves. The times were: MPPP, 30 min; alphaprodine, 30 min; morphine, 40 min; meperidine, 60 min. Doses (4—5) of each compound were tested, with 6—9 animals used at each dose.

In separate experiments, naive rats were injected with a single test compound and were qualitatively checked over a period of 90 min for signs typical of opiate intoxication. These consisted of: (1) bradykinesia, typically manifested by prolonged freezing episodes; (2) catalepsy, defined as the maintenance of unnatural, imposed postures; (3) loss of righting reflexes, as evidenced by the failure of an animal to immediatedly right itself when placed on its back; (4) loss of corneal (eye blink) reflexes; (5) respiratory depression. Two doses of each compound were tested, the dose which gave maximal analgesia and twice this dose. For MPTP, doses of 20 μM/kg, which produced a maximal facilitation of the tail flick reflex and 40 μM/kg were used.

RESULTS

The results of the binding experiments demonstrate that MPPP alone possesses appreciable opiate binding activity. Figure 1 indicates that MPPP has a higher affinity for the μ-sites occupied by DAGO than for the delta sites occupied by DADL. MPPP competed with naloxone when binding conditions favored agonist binding (Fig. 1C), but exhibited no inhibition of

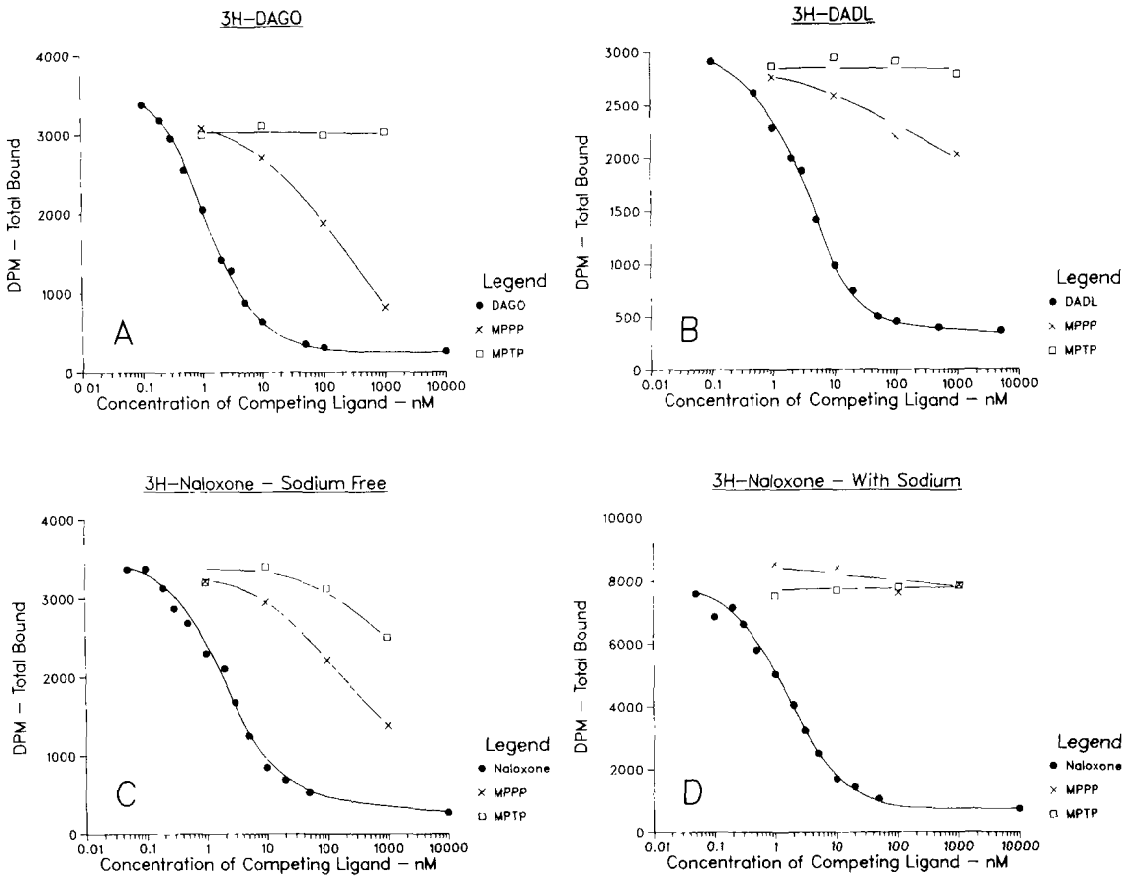

Fig. 1. Competition for binding sites between MPPP, MPTP and various opiate ligands. All tritiated ligands were present at a final-concentration of 1 nM. Incubations were at room temperature for 0.5 h in Tris buffer, 0.05 M (pH 7.4) with the exception of D, which was done at 0°C for 1 h in a Tris-Krebs buffer.

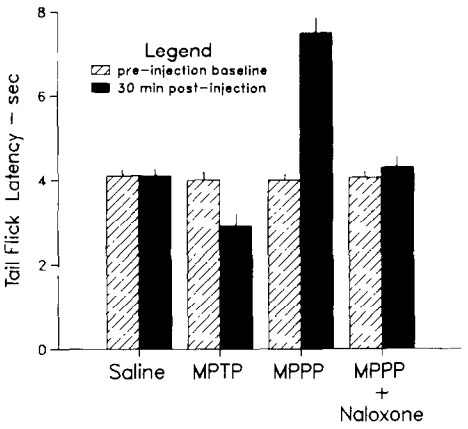

Fig. 2. Effect of saline (control), MPTP (20 μM/kg), MPPP (5 μM/kg), and a combination of MPPP and naloxone (both 5 μM/kg) on the rat tail flick latency. Striped bars indicate the average preinjection latency, solid bars the average latency 30 min after injection. MPTP caused a significant reduction in the tail flick latency ($P < 0.001$), while MPPP elevated the tail flick latency ($P < 0.001$). The elevation caused by MPPP was prevented by co-injection of naloxone. (Saline and MPTP groups, $n = 6$; MPPP and MPPP + naloxone groups $n = 12$).

naloxone binding under conditions which favor antagonist binding (Fig. 1D). This suggests MPPP is a pure agonist of primarily μ character. The weak inhibition of naloxone binding by MPTP under agonist conditions, but the lack of affinity for DAGO or DADL sites is curious and remains unexplained.

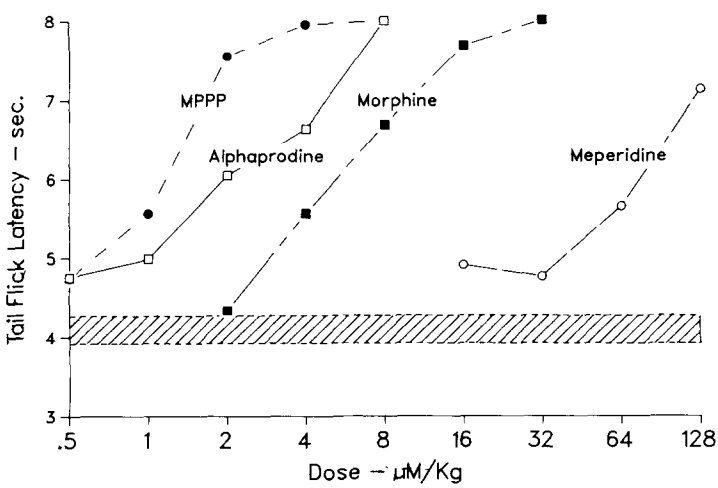

Fig. 3. Dose response curve of analgesic potency for MPPP compared to the related compounds alphaprodine and meperidine and the standard analgesic morphine. The striped bar indicates the preinjection range of tail flick latencies. ($n = 6$—9 for all groups).

TABLE I

APPEARANCE OF SIGNS OF OPIATE INTOXICATION AFTER INJECTION OF ALL TEST COMPOUNDS

Note that MPTP produced no signs typical of opiate action.

Compound	Dose μM/kg	Brady-kinesia	Catalepsy	Righting Reflex	Corneal Reflex	Resp. Depr.
MPPP	8	+	—	+	+	—
	16	+	+	—	—	—
MPTP	20	—	—	+	+	—
	40	—	—	+	+	—
Alphaprodine	8	+	—	+	+	—
	16	+	+	—	—	—
Morphine	16	+	—	+	+	—
	32	+	+	—	—	—
Meperidine	128	+	—	+	+	—
	256	+	+	—	—	+

The tail flick results (Fig. 2) confirmed the analgesic activity of MPPP, while revealing that MPTP has no analgesic effect and, in fact, causes a sensitization of the tail flick response. Higher doses of MPTP than that shown in Fig. 2 (40 μM/kg) did not lower the tail flick latency further and lower doses (10 μM/kg) did not effect the tail flick latency at all. As would be expected from the binding data, the elevation in tail flick latency caused by MPPP was prevented by the coinjection of an equimolar dose of naloxone.

MPPP proved to be an extremely powerful analgesic (Fig. 3) compared to other opiates, in accordance with prior results [4]. The potency of MPPP was 2 and 50 times that of the structurally related analogs alphaprodine and meperidine respectively and approx. 8 times that of morphine. In addition, classical signs of opiate intoxication were observed after high doses of morphine, meperidine, alphaprodine, and MPPP, but not MPTP (Table I). At doses of MPPP which yielded maximal analgesia, the more prominent sign was the episodic interruption of normal exploratory behavior by freezing. This bradykinesia could be reversed easily by a puff of air or some other means of startling the animal. Catalepsy, and respiratory depression were absent and corneal and righting reflexes were normal. At higher doses, catalepsy was observed, and righting and corneal reflexes were inhibited. Only the highest dose of meperidine produced dramatic respiratory depression (50 breaths/min as compared to 90—120 breaths/min observed in controls).

The relative proportions of MPPP and MPTP in street drugs are likely to vary widely, because MPTP is a by-product of MPPP synthesis. One confiscated drug preparation contained MPTP and MPPP in a ratio of 10:1.

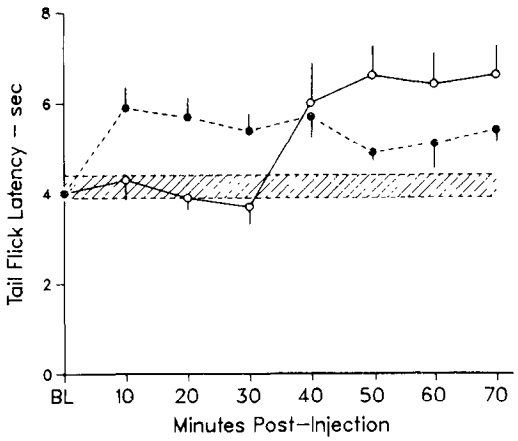

Fig. 4. Comparison of the analgesic effect of pure MPPP (2 μM/kg) and a 10 : 1 mixture of MPTP (20 μM/kg) and MPPP (2 μM/kg). The analgesic actions of MPPP were suppressed for 30 min, then suddenly expressed. Significant differences were seen between the two groups ($P < 0.05$) at 10, 20, 30 and 50 min after injection. The hatched area indicates the range of preinjection latencies. (For both groups, $n = 6$). ●, MPPP (2 μM/kg); ○, MPPP (2 μM/kg) + MPTP (20 μM/kg).

The effects of the combination of MPTP and MPPP in this ratio on the tail flick response provided an interesting result (Fig. 4). For the first 30 min after injection, the analgesia expected from MPPP was blocked or masked, presumably by the facilitative action of MPTP on the flection reflex. However, after 30 min, the analgesic action of MPPP was suddenly and dramatically expressed, yielding a degree of analgesia greater than that induced by an equal dose of MPPP alone.

DISCUSSION

The results demonstrate that the opiate effects of mixtures of MPTP and MPPP are due solely to MPPP. MPTP displayed no opiate activity across a variety of in vitro and in vivo tests. Conversely, MPPP was found to be an extremely potent naloxone reversible opiate agonist of predominant μ character. This observation fits well with subjective assessments of addicts who injected the MPTP-MPPP combination and described the effects to be most nearly like morphine, another μ agonist. Importantly, the opiate effects (at least analgesia) can be fully expressed even when taken in small doses and mixed with a 10-fold excess of MPTP.

The pharmacological nature of MPTP remains undefined. Previous experiments have shown that some of the acute effects of MPTP can be blocked by methysergide [10], suggesting direct or indirect stimulation of serotonergic receptors. This may explain the hallucinations sometimes experienced by users immediately after injection of the MPTP-MPPP mixture [2]. In addi-

tion, MPTP acts as a nicotinic agonist in a bovine adrenal chromaffin cell culture preparation, causing a dose related, hexamethonium reversible release of catecholamines (Hotchkiss et al., submitted for publication).

In conclusion, in vitro and in vivo assays of opiate activity demonstrate the opiate nature of MPPP while proving MPTP to be devoid of such properties. The high potency and morphine-like characteristics of MPPP, which are evident even when delivered in combination with a 10-fold excess of MPTP, are sufficient to explain repeated use of this combination by heroin addicts.

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