Pharmacology Biochemistry & Behavior, Vol 27, pp 457–461 © Pergamon Journals Ltd , 1987 Printed in the U S A 0091-3057/87 $3 00 + 00

Influences of Electrical Lesions of the Dopaminergic System on Morphine- and U-50,488H-Induced Analgesia in Rats

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Received 7 November 1986

OHNO, M , T YAMAMOTO AND S UEKI *Influences of electrical lesions of the dopaminergic system on morphine- and U-50,488H-induced analgesia in rats* PHARMACOL BIOCHEM BEHAV 27(3) 457–461, 1987 —The effects of electrical lesions of brain areas containing dopamine cell bodies and terminals on morphine analgesia were investigated and compared with those of a selective kappa-opioid agonist, U-50,488H The analgesic effect of morphine 10 mg/kg IP was potentiated significantly in substantia nigra (SN)- or caudate-putamen-lesioned rats, but not by ventral tegmental area (VTA) or nucleus accumbens lesions However, electrical lesions of neither SN nor VTA affected the analgesic activity of U-50,488H 32 mg/kg IP Although the tolerance to morphine analgesia developed in all four of the lesioned groups as well as in sham-lesioned rats, a significant analgesic effect in the SN-lesioned group prevailed during chronic treatment for 14 days as compared with that of sham-lesioned rats From these results, it is suggested that (1) morphine analgesia is potentiated by dysfunction of the nigro-striatal dopaminergic system, but not by that of the mesolimbic dopaminergic system, (2) the central dopaminergic system is not involved in the appearance of U-50,488H analgesia and is not basically related to the development of tolerance to morphine analgesia

Morphine Kappa-opioid agonist U-50,488H Analgesia Tolerance Dopamine Brain lesion Substantia nigra

DOPAMINERGIC antagonists potentiate morphine analgesia, while dopaminergic stimulants produce its inhibition [4, 8, 15, 17, 18] From these findings, it has been suggested that dopaminergic systems may be negative modulators of morphine analgesia However, others have reported opposite results, dopamine stimulants potentiate and antagonists block morphine analgesia [2, 10, 13] Clearly, studies on the effects of dopaminergic agents on morphine analgesia have produced inconsistent results Furthermore, there are few reports concerning the involvement of the dopaminergic system in the development of tolerance to morphine analgesia

On the other hand, a selective kappa-opioid agonist, U-50,488H, has been reported to display potent analgesic effects in mice, rats and rhesus monkeys [16,19] The antagonism of U-50,488H analgesia is far less sensitive to blockade by naloxone than that of morphine, whereas a reputed kappa-antagonist, MR-2266, is much more potent at blocking U-50,488H than morphine Furthermore, although tolerance occurs to U-50,488H analgesia, there is no cross-tolerance to morphine

In view of these findings, we investigated the effects of electrical lesions of brain areas containing cell bodies of dopamine neurons such as substantia nigra (SN), ventral tegmental area (VTA) and their terminals, caudate-putamen (CP), nucleus accumbens (NAc), on morphine analgesia in order to elucidate the functional differences of dopaminergic systems in the appearance of morphine analgesia Furthermore, the effects of these brain lesions on U-50,488H analgesia were compared with those of morphine so that the differential analgesic mechanisms of kappa- and mu-agonists might be clarified Whether or not the dopaminergic system is implicated in the development of morphine tolerance was also investigated

METHOD

Animals and Surgery

The animals used were male Wistar strain rats (body weight 250–300 g at the time of surgery) obtained from Shizuoka laboratory animal center They were housed in a room maintained at a temperature of 22±1°C with a 12 hr light-dark cycle (light period 07 00–19·00) Food and water were supplied ad lib throughout the experimental period

Rats were anesthetized with sodium pentobarbital (40 mg/kg IP) and fixed in a stereotaxic instrument For induction of bilateral electrical lesions, a stainless steel electrode (0 4 mm in diameter) insulated except 0 5 mm from the tip was inserted into the various brain areas, located according to the rat brain atlas of Paxinos and Watson [11] A direct

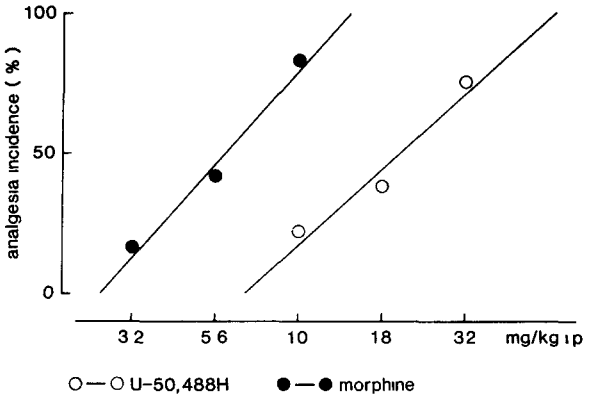

FIG 1 Analgesic effects of U-50,488H and morphine in the rat hot-plate test The animals whose hot-plate latency exceeded 20 sec were considered to be analgesic responders Each point represents the incidence of analgesia 0 5 hr after drug injection in each group of 8–12 rats

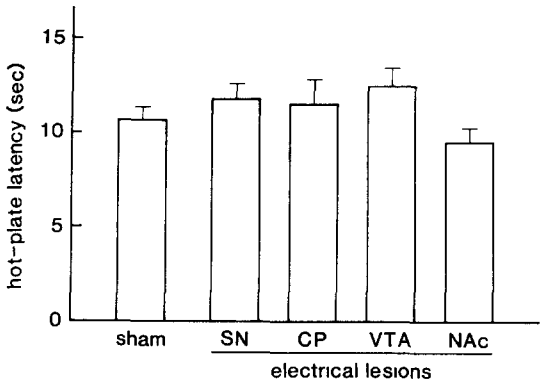

FIG 2 Influences of electrical lesions of the substantia nigra (SN), caudate-putamen (CP), ventral tegmental area (VTA) and nucleus accumbens (NAc) on pain sensitivity The hot-plate test was carried out prior to drug injection on the 7th day after surgery Each value is the mean±S E for 8–16 animals

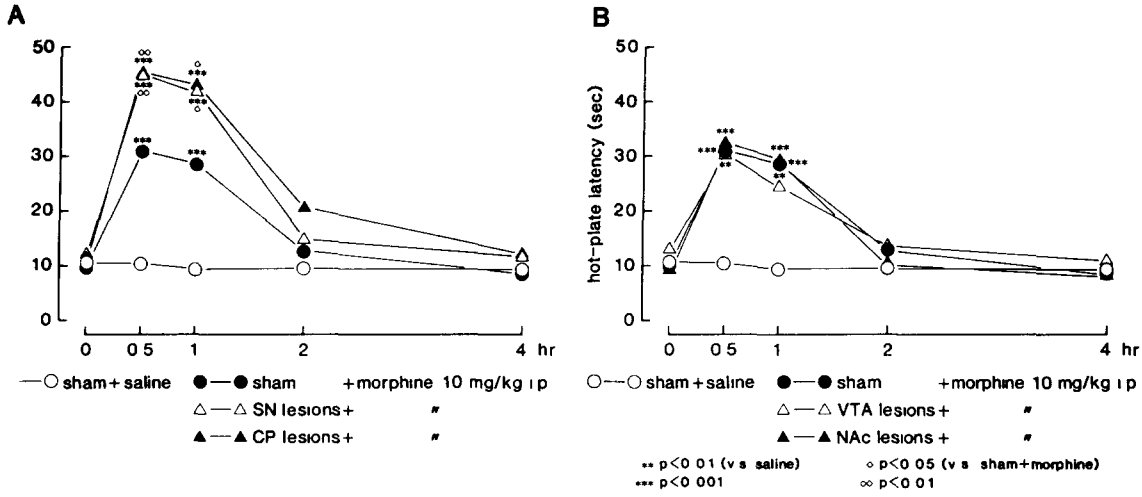

Fig 3 Influences of electrical lesions of the substantia nigra (SN), caudate-putamen (CP), ventral tegmental area (VTA) and nucleus accumbens (NAc) on morphine-induced analgesia Each point represents mean hot-plate latency for 8–16 animals Significant differences from the saline-injected group (**$p<0$ 01, ***$p<0$ 001) and from the morphine-injected group of sham lesions ($\diamond p<0$ 05, $\diamond\diamond p<0$ 01) were determined using Student's t-test

current of 2 mA was applied for 15 sec to destroy SN [anterior (A) 3 7 mm from the interaural line, lateral (L) 2 2 mm, horizontal (H) 8 6 mm], VTA (A 3 7 from the interaural line, L 0 8, H 9 0) and 3 mA for 20 sec to CP (rostral CP, A 1 5 from bregma, L 2 3, H 5 5, caudal CP, A 0 5, L 3 3, H 6 1), NAc (rostral NAc, A 2 0 from bregma, L 1 5, H 7 0, caudal NAc, A 1 0, L 1 7, H 7 7) Sham-lesioned rats were treated in the same way without applying the current after insertion of the electrode One week was allowed for recovery from the surgery before starting the experiment

Analgesic Assay

The hot-plate test was employed for the measurement of analgesic effects A rat was placed in a copper cylinder (40 cm in high, 25 cm in inner diameter) fixed in a water bath whose temperature was maintained at 55±0 5°C The time from contact with the plate until the occurrence of either hind-paw licking or jumping was recorded as the hot-plate latency When a rat produced neither licking nor jumping within 50 sec, the rat was immediately removed from the hot-plate and the latency was recorded as 50 sec The hot-plate test was carried out on the 7th day after surgery The analgesic test was performed just prior to and 0 5, 1, 2 and 4 hr after the drug injection Chronic administration was conducted at 12 00–15 00 once daily for 14 days and analgesic effects were measured on the 3rd, 7th and 14th days

Drugs

The drugs used in this study were morphine hydrochloride (Takeda) and U-50,488H (trans-3,4-dichloro-N-methyl-N-(2-(1-pyrrodinyl)-cyclohexyl)-benzeneacetamide methanesulfonate, hydrate, The Upjohn Company) Both drugs were dissolved in distilled water and administered intraperitoneally at 0 1 ml per 100 g body weight

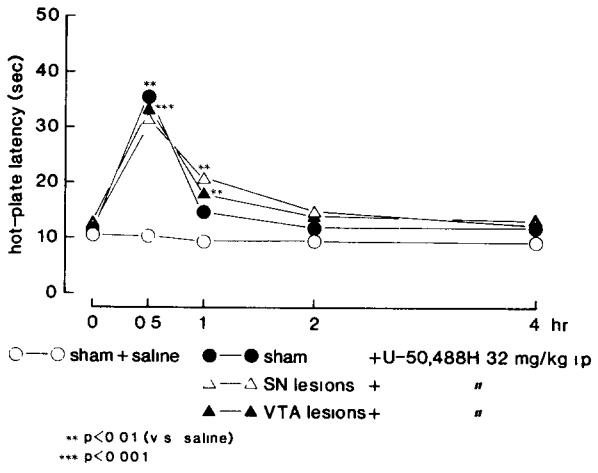

FIG 4 Influences of electrical lesions of the substania nigra (SN) and ventral tegmental area (VTA) on U-50,488H-induced analgesia Each point represents mean hot-plate latency for 8–16 animals Significant differences from the saline-injected group (Student's t-test), $^{**}p<0\ 01$, $^{***}p<0\ 001$

Histology

After completion of the experiment, the animal was anesthetized with ether and the brain was perfused with 10% formalin solution through the left cardiac ventricle After the brain was removed, sectioned slices were stained with cresyl violet The site and extent of the brain lesion were verified histologically Those animals who developed lesions in areas other than those under study were excluded

Statistical Analysis

The significance of analgesic effects was determined using the Student's t-test The ED_{50} values and their 95% confidence interval were calculated by the method of Litchfield and Wilcoxon [7]

RESULTS

Analgesic Effects of U-50,488H and Morphine

As shown in Fig 1, U-50,488H and morphine produced analgesic effects in a dose-dependent manner The analgesic potency of U-50,488H was approximately 1/3 that of morphine, the ED_{50} values of U-50,488H and morphine were 20 2 (12 9–31 6) mg/kg and 6 0 (4 5–7 9) mg/kg, respectively

Influences of Electrical Lesions of SN, CP, VTA and NAc on Morphine- and U-50,488H-Induced Analgesia

The histological examination revealed that electrical lesions of SN were teardrop shaped with a diameter of about 1 mm and exclusively localized in the pars compacta rather than in the pars reticulata of SN The VTA lesions were almost completely conducted (diameter about 1 mm) However, in animals with the maximal lesions of VTA, the destruction was extended to a part of the red nucleus and the superior cerebellar peduncle The lesions of CP and NAc were confined to partial destructions (diameter 1–1 5 mm) of these large structures

Pain sensitivity was not affected by electrical lesions of SN, CP, VTA and NAc (Fig 2) The hot-plate latency of saline-treated rats remained almost constant following the time-course of 0, 0 5, 1, 2 and 4 hr after administration Morphine at 10 mg/kg significantly increased the pain threshold at 0 5 and 1 hr after IP injection in sham-lesioned rats (Fig 3) Moreover, this analgesia was potentiated significantly in SN- or CP-lesioned rats (Fig 3A), but not by VTA or NAc lesions (Fig 3B)

U-50,488H at 32 mg/kg also produced a significant analgesic effect, whose potency was almost equipotent to that of morphine 10 mg/kg, but duration was shorter than that of morphine (Fig 4) Electrical lesions of neither SN nor VTA affected the U-50,488H analgesia

Influences of Electrical Lesions of SN, CP, VTA and NAc on the Development of Tolerance to Morphine Analgesia

Chronic treatment with saline for 14 days did not alter the hot-plate latency When morphine at 10 mg/kg was administered once daily for 14 days in sham-lesioned rats, morphine analgesia was diminished gradually compared with that of the 1st injection and disappeared completely on the 7th and 14th days (Fig 5) Although the diminution of morphine analgesia by chronic treatment was also observed in all four lesioned groups, the analgesic effect in SN-lesioned group was prolonged and lasted through the 7th and 14th days (Fig 5A)

The analgesic effect produced by morphine 5 6 mg/kg was also potentiated significantly by SN lesions In that case, the potency was almost equipotent to that of morphine 10 mg/kg in sham-lesioned group (Fig 6) Unlike the effect of the 10 mg/kg dose, the potentiated analgesia of morphine at 5 6 mg/kg in SN-lesioned rats was significantly reduced and disappeared completely after chronic treatment for 7 days

DISCUSSION

The analgesic effect of morphine in rats was potentiated significantly by SN- or CP-lesions in spite of the lack of significant alterations of pain sensitivity by itself In mice, the electrical lesions of caudate nucleus produced a persistent potentiation of morphine-induced analgesia in escape latency from foot shock [3] Conversely, electrical lesions of neither VTA nor NAc influenced the analgesic activity of morphine Meanwhile, neuroleptics such as haloperidol, pimozide and spiroperidol also produce potentiation of morphine analgesia, which results from blockade of dopaminergic receptors [4, 8, 15, 17, 18] From these results, morphine analgesia is potentiated by dysfunction of the nigro-striatal dopaminergic system, but not by that of the mesolimbic dopaminergic system However, it is conceivable that there is fundamentally no direct involvement of the central dopaminergic system in the appearance of morphine analgesia since the lesions of the dopaminergic system failed to abolish the analgesic activity Szreniawski *et al* [14] also report that ventral tegmental dopamine neurons are not primarily involved in the analgesic activity of morphine measured by a tail pressure method in rats Although their findings give support to the present results, they differed by the fact that VTA lesions decreased the pain threshold in their experiments

On the other hand, CP has been known to send the projections of γ-aminobutyric acid (GABA)-containing neurons to SN [6] Therefore, there is a possibility that the potentiation of morphine analgesia in CP- or SN-lesioned rats may result from the alteration of the GABAergic system Actually, it has been shown that GABAergic stimulant drugs an-

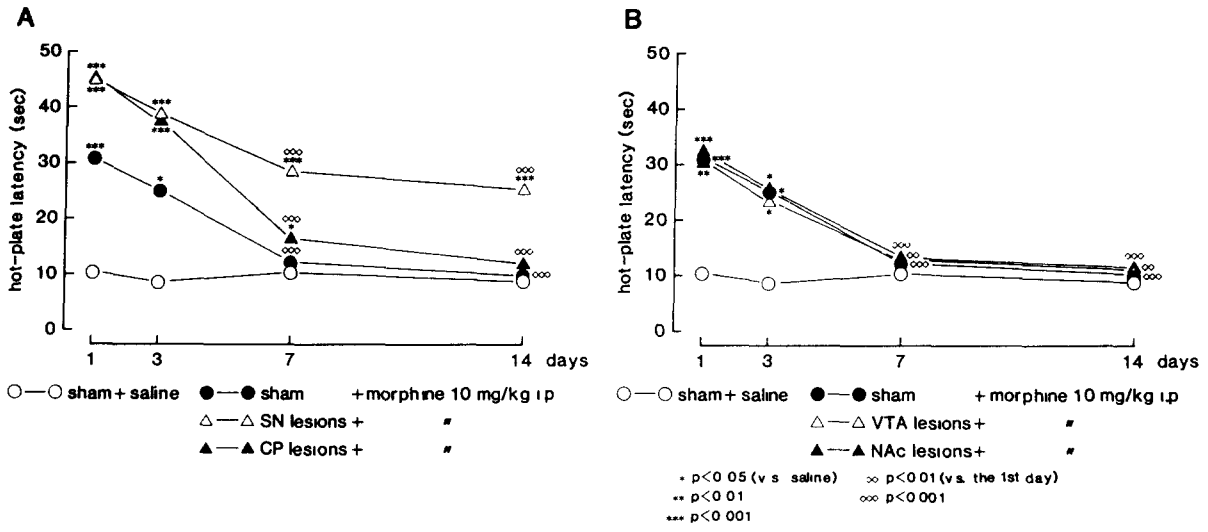

FIG 5 Influences of electrical lesions of the substantia nigra (SN), caudate-putamen (CP), ventral tegmental area (VTA) and nucleus accumbens (NAc) on the development of tolerance to morphine analgesia Morphine 10 mg/kg or saline was injected once daily for 14 days Each point represents mean hot-plate latency 0 5 hr after drug injection in each group of 6–16 animals Significant differences from the saline-injected group (*$p<0$ 05, **$p<0$ 01, ***$p<0$ 001) and from the values of the 1st day (∞ $p<0$ 01 ∞∞ $p<0$ 001) were determined using Student s t-test

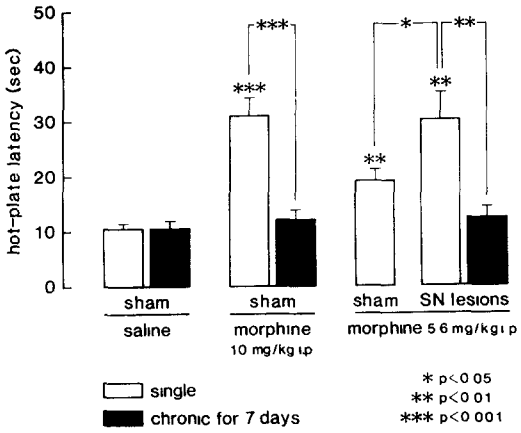

FIG 6 The disappearance of analgesic effect induced by morphine 5 6 mg/kg by chronic treatment in substantia nigra (SN)-lesioned group Each value is the mean hot-plate latency (±S E) 0 5 hr after drug injection in each group of 6–16 animals The significance of the effects of treatments was determined using Student's t-test *$p<0$ 05 **$p<0$ 01, ***$p<0$ 001

tagonize morphine-induced analgesia in rats [9] and mice [5] However, conversely, Biggio *et al* [1] reported that a GABA agonist, muscimol, potentiates morphine analgesic activity evaluated by the hot-plate method in mice These controversial findings make it difficult to understand the potentiation of morphine analgesia in this experiment from a viewpoint of the GABAergic system at present

The analgesic potency of the kappa-opioid agonist, U-50,488H, was approximately 1/3 that of morphine in the hot-plate test, which is similar to the results obtained by Von Voigtlander *et al* [19] Electrical lesions of either SN or VTA failed to affect the analgesic effect of U-50,488H unlike morphine analgesia These results suggest that the ascending dopaminergic neurons may not be involved in the analgesic action of U-50,448H Piercey *et al* [12] indicated that the locus of U-50,488H analgesic action may be in the spinal cord since U-50,488H produced analgesic effect when injected intraspinally, but was almost inactive after intracranial injection

Tolerance to the analgesic effect of morphine 10 mg/kg developed in all four lesioned groups as well as in sham-lesioned group However, the significant analgesic activity in SN-lesioned rats was markedly prolonged and lasted during chronic treatment for 14 days On the other hand, the potentiated analgesic activity of morphine 5 6 mg/kg in SN-lesioned rats, which was almost equipotent to that of morphine 10 mg/kg in sham-lesioned group, disappeared completely during chronic administration Therefore, a significant morphine analgesia observed on the 7th and 14th days in the SN-lesioned group was due to the potentiated analgesic effect of morphine on the 1st day In conclusion, it is conceivable that the central dopaminergic system may not be involved in morphine tolerance, although the development of tolerance to morphine analgesia tends to be delayed in SN-lesioned rats

ACKNOWLEDGEMENTS

The authors would like to acknowledge the valuable comments of Prof Dr R E Counsell (University of Michigan) We are also indebted to Dr R D Hudson (The Upjohn Company, Kalamazoo, MI) for the gift of U-50,488H, to Miss T Takaki for her technical assistance and to Miss H Hitaka for typing the manuscript

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