Pharmacology Biochemistry & Behavior, Vol. 25, pp. 959–965, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Opiate-Induced Turning in Rats After Injection Into the Ventral Tegmental Area[1]

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Received 31 December 1985

SZEWCZAK, M. R. AND M. T. SPOERLEIN. *Opiate-induced turning in rats after injection into the ventral tegmental area.* PHARMACOL BIOCHEM BEHAV **25**(5) 959–965, 1986.—Morphine and ethylketazocine caused ipsilateral circling when injected unilaterally into the ventral tegmental area (VTA) of rats. Systemic naloxone only slightly inhibited this effect while systemic diprenorphine completely prevented circling. Systemic haloperidol and alpha-methyl-p-tyrosine also blocked circling. Rats made tolerant to morphine still turned after morphine injection into the VTA. Levorphanol, dextrorphan, methadone, DADLE, dynorphin(1–13), SKF 10,047 and phencyclidine were inactive when injected unilaterally into the VTA of naive rats; naloxone and naltrexone alone also were inactive. The opiate-induced circling appears to involve a non-mu opiate receptor as well as a dopaminergic neuronal system.

Opiate-induced circling Mesolimbic dopamine Ventral tegmental area

THE effects of opiates on circling behavior have been examined by a number of investigators (for review see [34]). Direct unilateral injection of morphine, beta-endorphin, methadone, levorphanol, pentazocine, met-enkephalin, d-ala(2)-met-enkephalin and nalorphine into the substantia nigra of naive rats all induced continuous tight circling contralateral to the side of the injection, while naloxone, naltrexone and dextrorphan administered unilaterally to this site had no effect [17, 19, 23]. Haloperidol pretreatment or the destruction of the ascending nigrostriatal dopamine pathway on the same side as the injection blocked circling behavior seen with morphine [17,23].

Direct bilateral injections of morphine or beta-endorphin into the substantia nigra of naive rats resulted in intense stereotypic licking, biting, gnawing and sniffing which is blocked by systemically administered naloxone [17, 21, 23]. These studies suggest that the stimulation of nigral opiate receptors results in enhanced nigrostriatal transmission causing an increased release of dopamine and subsequent stereotypy and hyperactivity [18,31].

While studying the effects of direct injections of opiates into various rat brain regions, it was observed in this laboratory that morphine administered unilaterally into the ventral tegmental area (VTA) caused ipsilateral circling. Several other investigators, working in the VTA, have shown that bilateral administration of morphine, beta-endorphin, d-ala(2)-met-enkephalin or d-ala(2)-d-leu(5)-enkephalin caused naloxone and neuroleptic sensitive motor stimulation without stereotypy [4, 21, 22, 24, 37]. Since the VTA contains the A10 dopamine cell bodies which innervate the nucleus accumbens, olfactory tubercle, prefrontal cortex, and other structures and since circling behavior caused by opiate administration into the substantia nigra has an underlying dopaminergic mechanism [16,22], studies were designed: (1) to compare the effects of morphine administration into the VTA with those seen after administration into the substantia nigra seen by Iwamoto and Way [17] and Kaakkola [23]; (2) to study the involvement of the dopaminergic system in the actions of morphine in the VTA; and (3) to determine the type of opiate receptor which is responsible for the actions of morphine in the VTA.

METHOD

Male Wistar rats (Charles River), 180 to 250 grams, were used in these experiments. The animals were housed for at least 72 hours in a climate-controlled vivarium (23 degrees C, 50% relative humidity) with a 12 hour light/dark cycle (6 a.m./6 p.m.). Food and water were available at all times.

The following agents were utilized: d-Ala(2)d-leu (5)-enkephalin (DADLE, Calbiochem-Behring), dextrorphan tartrate (Hoffmann-LaRoche), diprenorphine HCl (NIDA), dynorphin(1–13) (Sigma), ethylketazocine methanesulfonate (Sterling-Winthrop), haloperidol (McNeil), levorphanol

[1]Portions of these data were presented at the Society for Neuroscience meetings at Boston, MA (1983) and Anaheim, CA (1984).

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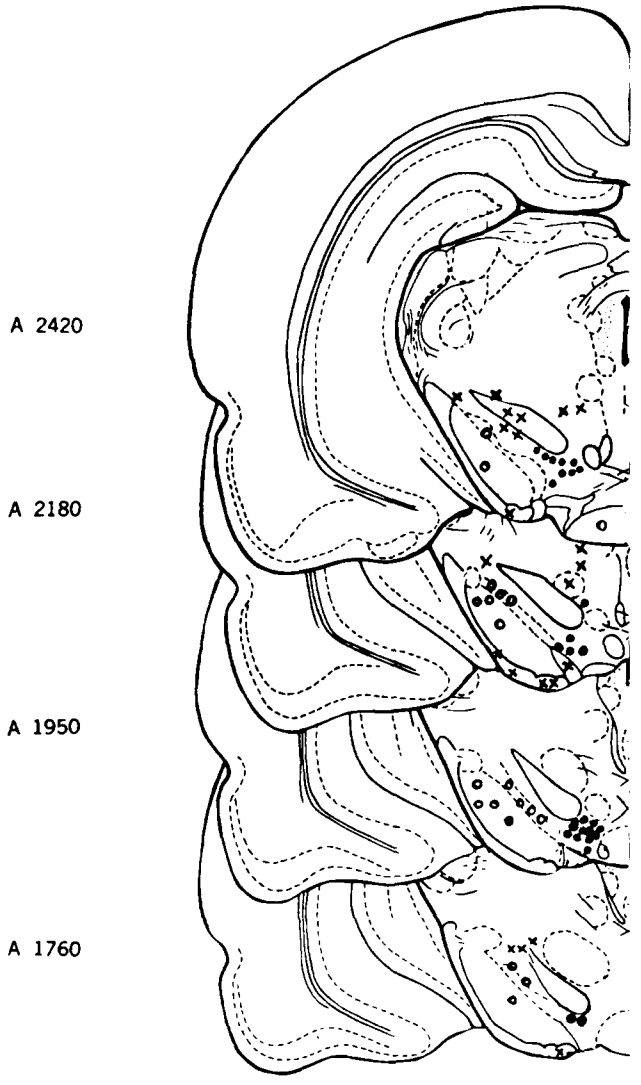

FIG. 1. Histological reconstruction of morphine injection site. Closed circles indicate sites causing ipsilateral circling, open circles indicate sites causing contralateral circling, crosses indicate sites which did not result in circling. Morphine doses were 25 or 50 nmoles, n=76.

bitartrate (Hoffman-LaRoche), methadone HCl (Lilly), alpha-methyl-p-tyrosine (Sigma), morphine sulfate (Merck), SKF 10,047 (racemic N-allyl-N-normetazocine, NIDA), naloxone HCl (Endo), naltrexone HCl (Endo), and phencyclidine HCl (NIDA).

Compounds administered intracerebrally were dissolved in 0.9% saline immediately before injection. To serve as controls, groups of rats were injected unilaterally into the VTA with 0.9% saline (1 microliter) or sodium sulfate (25 nmoles, control for morphine sulfate). Compounds administered systemically were adjusted for percent base and dissolved in 0.9% saline or, if insoluble, suspended in 0.9% saline with one drop of "Tween 80" surfactant added for each 5 ml. Suspensions were kept constantly agitated before administration. Morphine pellets were prepared using the method of Gibson and Tingstadt [10] modified so that each pellet contained 50 mg of morphine base.

For the acute studies compounds were administered intraperitoneally either 15 minutes (naloxone or diprenorphine), 30 minutes (haloperidol) or 4 hours (alpha-methyl-p-tyrosine) prior to an intracerebral injection. The test drug or 0.9% saline (control) was injected at a dosage volume of 1 ml/kg. In the chronic studies, two 50 mg morphine pellets (total of 100 mg of morphine base) were implanted subcutaneously in several experimental groups under light halothane anesthesia 76 hours prior to intracerebral injections. The pellets were removed from some animals 4 hours before intracerebral injections (withdrawal group), while the pellets were left in place in other animals (tolerance group) during the injections and behavioral measurements. After the pretreatment interval had elapsed, each rat was anesthetized using halothane, mounted in a stereotaxic frame (Kopf Model 900), and the skull exposed. Anesthesia was maintained throughout the surgery by placement of halothane-soaked swabs approximately 5 mm from the nose of the rat. Injection coordinates for the ventral tegmental area were A 2180, L 0.8 and V −2.8 according to the atlas of König and Klippel ([27]; see Fig. 1). Intracerebral injections were made in a volume of one microliter injected over one minute through a Hamilton 10 microliter syringe with a 28 gauge, nonbeveled injection needle. The injection needle was left in place an additional minute to allow diffusion of the substance injected. The incision was then closed, anesthesia was removed, and the rat was placed in a rotometer (Columbus Instruments). Since opiates were administered into the brain only once in order to avoid confounding problems of tolerance, hypersensitivity, and physical lesions, implanted chronic cannulae were not utilized.

All animals recovered from anesthesia with ten minutes. Fifteen minutes after the needle was removed, the behavioral session was started and complete turns were recorded and printed every fifteen minutes for 7 consecutive periods. At the end of the test session, the rats were sacrificed using halothane; the brains were removed, sectioned, and carefully examined for placement of the needle track tips. Only data from rats with injection needle tracks within the VTA were included in the calculations. The mean number of turns and the standard error for each group were calculated. The data were also transformed to the log(10) value for statistical analysis to normalize the variances and analyzed at each time point for significance from the controls, using a computerized analysis of variance procedure for unequal group sizes (GLM [36]). When a significant F value was found, a Fisher's *t*-test was performed to test for individual group significance.

Several rats from various drug groups were removed from their rotometers after their circling behavior had been assessed for 15 minutes and were observed for catatonia (rigid extension of the limbs), and for catalepsy (the ability to maintain the forelimbs over a horizontal bar for more than thirty seconds, in the absence of catatonia) [8].

RESULTS

Figure 1 shows the histological reconstruction of the cannula tip placements of 76 rats injected with morphine. Careful examination of cannula tip placement shows that rats injected in the VTA consistently circled ipsilateral to the site of injection, while injection in the substantia nigra caused contralateral circling. Placement of morphine dorsal to the VTA or the substantia nigra failed to cause circling. Neither 0.9% saline nor sodium sulfate alone caused circling when iniected into the VTA. Animals from all the treatment groups

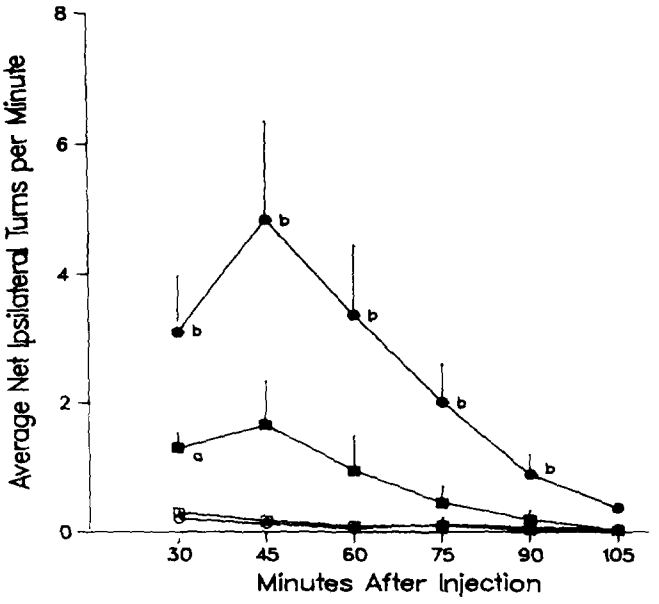

FIG. 2. Ipsilateral circling after unilateral VTA injection of morphine in rats. Doses below 12.5 nmoles were inactive. a: Significant at 0.05 level, b: significant at 0.01 level vs. VTA saline injected controls. N=8 for 50 nmole group, n=7 for 25 nmole group, n=7 for 12.5 nmole group, and n=16 for the control group. (●) Morphine sulfate 50 nmoles; (■) morphine sulfate 25 nmoles; (○) morphine sulfate 12.5 nmoles; (□) 0.9% saline 1 microliter.

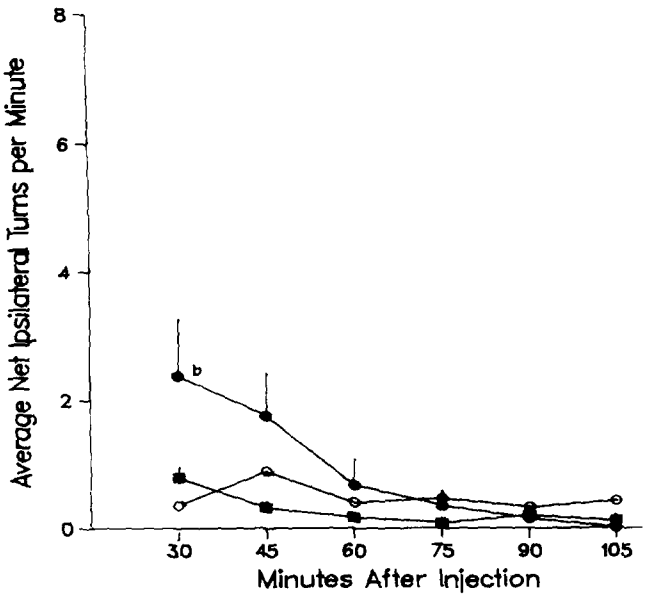

FIG. 3. Ipsilateral circling after unilateral VTA injection of kappa agonists in rats. a: Significant at 0.05 level, b: significant at 0.01 level vs. VTA saline group. N=7 for EKC at 10 nmoles, n=5 for EKC at 5 nmoles, n=2 for dynorphin(1–13), and n=16 for the control group. (●) Ethylketazocine 10 nmoles; (■) ethylketazocine 5 nmoles; (○) dynorphin(1–13) 50 nmoles.

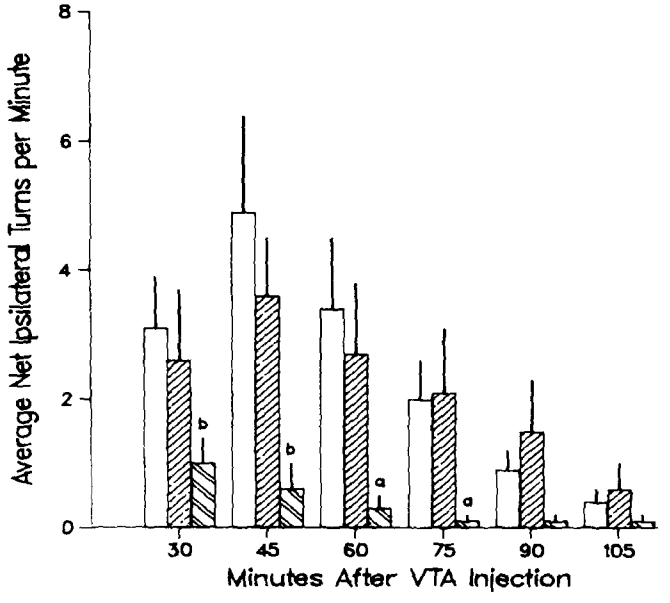

FIG. 4. Effects of narcotic antagonists on ipsilateral circling induced by VTA injections of morphine (50 nmoles). Naloxone administered simultaneously with morphine into the VTA. Systemic pretreatment with naloxone (10 mg/kg, IP) caused barrel rolling behavior when morphine was injected into the VTA. a: Significant at 0.05 level, b: significant at 0.01 level vs. morphine (50 nmoles) alone group. N=8 for morphine alone, n=7 for naloxone group, n=4 for diprenorphine group. Open column: morphine 50 nmoles; narrow striped column: plus naloxone 50 nmoles; wide striped column: plus diprenorphine 10.0 mg/kg.

with cannula tip placements outside of the VTA were not included in the analysis.

Figure 2 shows that unilateral injections of morphine into the VTA produced spontaneous ipsilateral circling behavior. The intensity of the turning was dose-related and at the highest (25–50 nmoles) doses the peak effect was at 30–45 minutes after injection. Turns were tight head-to-tail and occurred intermittently with brief periods of no turns. Marked postural asymmetry was seen ipsilateral to the side of injection during the periods of no circling. We attempted to trigger circling episodes [20] by auditory (hand clap), visual (sudden movement of a white and black target circle 5 cm in diameter within the visual field of the rat), or light tactile (air puffs). No alteration in circling was observed after any stimulation in morphine treated rats. We also failed to observe any catalepsy or catatonia in morphine injected rats. Doses of 0.7 to 12.5 nmoles of morphine caused no turning.

A number of other opiate agonists and antagonists were injected unilaterally into the VTA and the animals were observed for turning behavior. Figure 3 shows the effect of kappa agonists. Ethylketazocine caused significant turning at 10 nmoles but was inactive at 5 nmoles. Higher concentrations were insoluble. The turning was similar to that seen with morphine, a tight head-to-tail spontaneous circling. Dynorphin(1–13) at 50 nmoles failed to cause turning when injected unilaterally into the VTA.

The following opiate compounds did not cause circling. The sigma agonists SKF 10,047 (50 nmoles) and phencyclidine (50 nmoles) and the delta agonist DADLE (10 and 50 nmoles) did not cause circling or postural asymmetry. DADLE injected animals were all catatonic for the first 30 minutes after injection. The lipophilic mu agonist methadone (50 nmoles) also failed to cause turning. Levorphanol (25 and

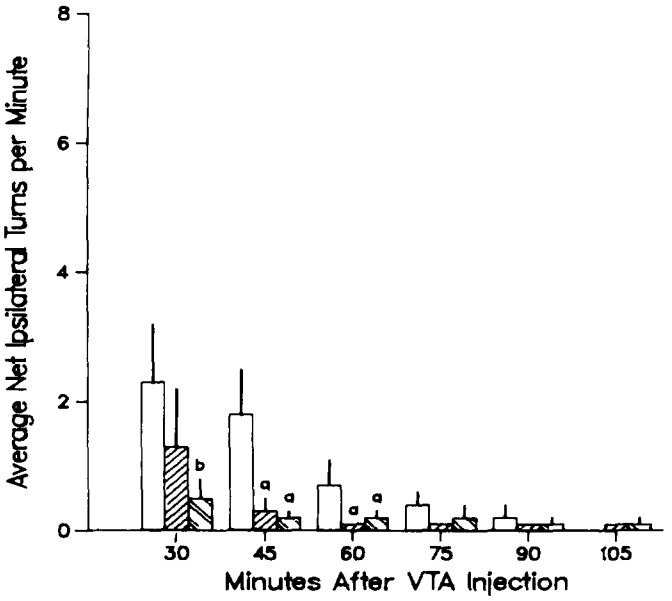

FIG. 5. Effects of narcotic antagonists on ipsilateral circling induced by unilateral VTA injection of ethylketazocine (10 nmoles). a: Significant at 0.05 level, b: significant at 0.01 level vs. ethylketazocine alone group. N=7 for ethylketazocine alone, n=4 for naloxone, n=4 for diprenorphine. Open column: ethylketazocine 10 nmoles; narrow striped column: plus naloxone 10.0 mg/kg; wide striped column: plus diprenorphine 10.0 mg/kg.

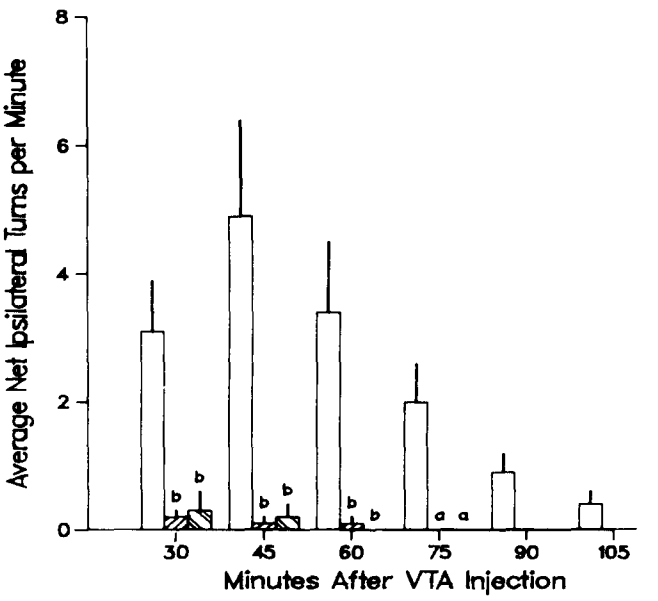

FIG. 6. Effects of alpha-methyl-p-tyrosine and haloperidol on ipsilateral circling induced by unilateral VTA injection of morphine (50 nmoles). a: Significant at 0.05 level, b: significant at 0.01 level vs. morphine alone group. N=8 for morphine alone, n=4 for alpha-methyl-p-tyrosine, n=5 for haloperidol. Open column: morphine 50 nmoles; narrow striped column: plus alpha-methyl-p-tyrosine 250.0 mg/kg; wide striped column: plus haloperidol 0.5 mg/kg.

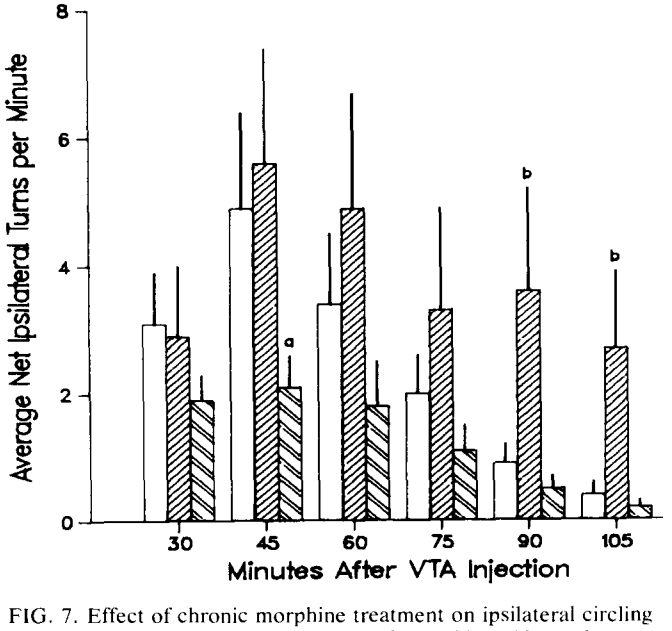

FIG. 7. Effect of chronic morphine treatment on ipsilateral circling induced by unilateral VTA injection of morphine (50 nmoles). a: Significant at 0.05 level, b: significant at 0.01 level vs. VTA morphine alone group. N=8 for VTA morphine alone, n=4 for pellets in, n=4 for pellets out. Open column: morphine 50 nmoles; narrow striped column: plus pellets in; wide striped column: plus pellets out.

50 nmoles), a mu agonist, was tested; the rats did not turn but all rats displayed catalepsy without muscle rigidity. Its stereoisomer dextrorphan, which is essentially devoid of mu agonist activity, caused neither turning nor catalepsy. The antagonists naloxone (25 and 50 nmoles) and naltrexone (50 nmoles) failed to cause turning.

Figures 4 and 5 show the effects of narcotic antagonists on opiate-induced ipsilateral turning. Naloxone (50 nmoles) injected simultaneously with morphine (50 nmoles) into the VTA did not block the ipsilateral turning behavior (Fig. 4). Pretreatment with naloxone at 10 mg/kg IP, 15 minutes prior to VTA morphine injection, resulted in marked barrel rolling with periods of intense ipsilateral turning after 50 nmoles of morphine. The same pretreatment had no effect on circling caused by morphine at 25 nmoles. The opiate antagonist diprenorphine at 10 mg/kg IP, 15 minutes prior to morphine injection into the VTA at 50 nmoles, inhibited turning behavior (Fig. 4). Figure 5 shows that naloxone (10 mg/kg IP, 15 minutes pretreatment) significantly reduced ethylketazocine-induced turning at 45 and 60 minutes. The relevance of this finding may be slight, since the degree of turning seen in the ethylketazocine alone group was not significantly different from VTA-saline control levels at these time points. Simultaneous administration of naloxone (50 nmoles) with ethylketazocine (10 nmoles) in the VTA did not inhibit turning. Diprenorphine pretreatment (10 mg/kg IP, 15 minutes) completely blocked ethylketazocine-induced ipsilateral turning at 30 minutes (Fig. 5).

Alpha-methyl-p-tyrosine (250 mg/kg IP, 4 hour pretreatment) completely blocked morphine-induced circling from the VTA as did the dopamine antagonist haloperidol (0.5 mg/kg IP, 30 minutes pretreatment; Fig. 6). The effects of chronic morphine treatment on turning caused by morphine injection into the VTA appear in Fig. 7. In the group that

retained the pellet during VTA injection (tolerance group), morphine caused the same degree of turning as that seen in naive rats at earlier time points, with significantly increased turning at later time points. In the group that had the pellets removed (withdrawal group), a significant decrease in circling was seen overall using a repeated measures analysis of variance, with the 45 minute group contributing significantly ($p<0.05$) to the difference. These animals all displayed teeth-chattering, wet dog shakes, and diarrhea.

DISCUSSION

The three characteristics of opiate-induced turning from the substantia nigra are contralateral direction, sensitivity to mu opiate receptor antagonists, and the high degree of tolerance seen after chronic morphine administration [17,23]. The studies described in this paper demonstrate opiate-induced circling mediated by the VTA with fundamentally different characteristics, namely ipsilateral direction, little or no sensitivity to mu receptor antagonists and little or no tolerance after chronic morphine administration.

In contrast to our findings, contralateral turning behavior elicited by morphine placement in the VTA was reported by Holmes and coworkers [12–14]. The characteristics of this circling closely match the circling seen by Iwamoto and Way [17] in the substantia nigra. This may be explained by differences in technique. Holmes and coworkers applied crystalline morphine through chronic cannulae to the VTA and found naloxone sensitive contralateral circling around the perimeter of an open field which increased over the two hour session and over the four days of application. They also report that in several of these animals, they subsequently administered 5 micrograms of morphine dissolved in 0.5 microliters which also resulted in contralateral circling. In an unpublished study, we similarly prepared rats with chronic cannulae in order to address the discrepancy between our results and those of Holmes *et al.* We found that if morphine was first infused as a solution, ipsilateral circling resulted. Subsequent application of crystalline morphine caused contralateral circling. We applied crystalline morphine for 4 days, and then a week later we infused morphine solution once again. This time only contralateral circling was seen. Based on these observations, we feel that the phenomena observed by Holmes and coworkers may be the result of specific changes induced by the experimental technique employed in their studies (see [3,30]) which activates a specific population of neurons and which masks the ipsilateral circling seen in our study.

Because the ipsilateral circling caused by morphine is blocked by alpha-methyl-p-tyrosine and haloperidol, it is tempting to explain any postural asymmetry and circling resulting from drug placement in the VTA as being due to mechanisms similar to those found in the nigrostriatal dopamine system, namely that contralateral circling resulted from stimulation of dopamine transmission while ipsilateral circling results from inhibition of dopamine transmission [34]. Since stimulation of motor activity by various dopamine agonists injected bilaterally into mesolimbic terminal regions results in increased motor activity [9, 25, 32], lesions of these areas cause hypoactivity [25,28], and unilateral stimulation of mesolimbic dopamine neurons which do not bilaterally innervate the terminal fields such as the olfactory tubercles causes contralateral circling [38], it could be inferred that morphine and ethylketazocine appear to inhibit the firing of A10 dopamine neurons, at least in opiate naive rats.

Jacquet *et al.* [20] observed ipsilateral turning after unilateral injection of opiates into the mesencephalic reticular formation. This turning was insensitive to naloxone and to catecholaminergic blockade. This region is believed to be part of the output pathway from the basal ganglia [28,33]. However, the turning seen from this site was not spontaneous and was provoked by external auditory or visual stimulation. This is in contrast to the spontaneous circling seen in our studies after unilateral injection of morphine into the VTA; this circling was sensitive to catecholaminergic blockade. External sound or visual stimulation had no effect on the VTA-mediated turning behavior.

Ipsilateral turning due to unilateral administration of morphine into the VTA was not blocked by systemic (10 mg/kg) or simultaneous direct naloxone injection into the VTA (50 nmoles), which is markedly different from the complete inhibition of opiate-induced turning from the substantia nigra [17,23]. Diprenorphine completely blocked opiate-induced turning from the VTA. Since diprenorphine serves as an antagonist with approximately equal affinity for mu, kappa and sigma opiate receptors while naloxone has a much greater affinity as an antagonist for mu receptors [32], these data would suggest that the circling behavior seen after morphine administration into the VTA is probably due to stimulation of a non-mu opiate receptor and not the result of a nonopiate effect. Although naloxone does interact with other non-mu opiate receptors (with low affinity), it did not effectively block the morphine or ethylketazocine-induced ipsilateral circling. We have no explanation for this lack of effect.

An examination of the effects of chronic morphine on morphine-induced ipsilateral circling from the VTA shows no tolerance to morphine after 3 days of continuous exposure. The lack of tolerance is in direct contrast to the marked tolerance seen with chronic morphine in morphine-induced contralateral circling from the substantia nigra [17]. Some potentiation of ipsilateral circling was seen, which may reflect tolerance at mu receptors that may mediate the contralateral circling seen by Holmes *et al.* [12–14]. The result would be a more complete expression of non-mu opiate receptor mediating ipsilateral circling. In animals undergoing spontaneous withdrawal, a decrease in circling was seen. The significance of this decrease during withdrawal is not clear at this time; it may simply reflect nonspecific behavioral impairment caused by the withdrawal state.

Of the other opiates, besides morphine, tested for their ability to cause turning after unilateral injection into the VTA, only ethylketazocine caused ipsilateral circling. The turning caused by this kappa agonist [5] was slightly but significantly inhibited by naloxone and completely blocked by diprenorphine. The mu agonists levorphanol and methadone, the delta agonist d-ala(2)-d-leu(5)-enkephalin and the sigma agonists SKF 10,047 and phencyclidine [5,40] all failed to cause turning when administered unilaterally into the VTA. This is different from the effects reported for nigral administration of these compounds. Morphine, levorphanol and methadone all caused contralateral turning and when naloxone was systemically administered, morphine-induced turning was completely antagonized [17,23].

Dynorphin(1–13), a fragment of the endogenous opioid dynorphin(1–17) has been reported to be a relatively specific kappa agonist [7, 15, 35, 39]. In our hands this peptide was devoid of circling activity. However, since it is likely that this fragment is very rapidly metabolized to inactive fragments [6, 11, 15, 37], the lack of activity 15 minutes after

injection may not reflect its true behavioral potential.

To briefly summarize, morphine and ethylketazocine caused ipsilateral circling behavior when injected unilaterally into the VTA. Other opiates tested were without effect. The opiate-induced turning is possibly mediated by a non-mu receptor based on a comparison of inactive agonists, antagonists and tolerance characteristics, and appears to be dopamine-dependent.

ACKNOWLEDGEMENTS

The authors would like to thank Endo Laboratories, Hoffmann-LaRoche Inc., Eli Lilly and Co., McNeil Pharmaceuticals, Merck and Co., the National Institute for Drug Abuse and Sterling-Winthrop for the generous gifts of drugs used in these studies. The authors also wish to thank Hoechst-Roussel Pharmaceuticals Inc. for the use of their facilities. These studies were supported in part by a Charles and Johanna Busch Research Grant from Rutgers University.

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