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Facilitation of Self-Stimulation Behavior Following Intracerebral Microinjections of Opioids Into the Ventral Tegmental Area

C. L. BROEKKAMP[1] AND A. G. PHILLIPS

Department of Psychology, University of British Columbia, Vancouver, Canada

AND

A. R. COOLS

Department of Pharmacology, University of Nijmegen, The Netherlands

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BROEKKAMP, C. L., A. G. PHILLIPS AND A. R. COOLS. *Facilitation of self-stimulation behavior following intracerebral microinjections of opioids into the ventral tegmental area.* PHARMAC. BIOCHEM. BEHAV. 11(3) 289–295, 1979.—The intracerebral microinjection technique was used to localize sites in the brain where morphine facilitated the self-stimulation rate at hypothalamic electrode sites. Bilateral injections of morphine (2×1 μg) into the ventral tegmental area and substantia nigra produced the strongest enhancement at the shortest latencies. At these sites, bilateral injections of 200 ng of morphine also produced a significant enhancement whereas a dose of 50 ng was below threshold for the rate increasing effect. The enhancement by morphine was effectively antagonized by naloxone (5 mg/kg). When injected bilaterally into the same area, D-Ala^2-Met^5-enkephalinamide (2×1 μg) also induced a strong enhancement of self-stimulation lasting for 70 minutes. A possible dopaminergic substrate for the opiate induced behavioral stimulation is discussed.

Self-stimulation Morphine Enkephalin Naloxone Ventral tegmental area Rat

SHORTLY after the discovery of intracranial self-stimulation (ICS) it was suggested that this preparation may be helpful in understanding the psychological properties of addictive drugs [27]. Morphine was among the first drugs studied on ICS behavior, and on initial investigation revealed [28] a predominantly inhibitory effect with septal and hypothalamic electrodes and some facilitation with ventral tegmental placements. The locus specific effects of morphine have been emphasized further in several recent papers [15, 20, 23], thus raising the possibility that the depressant and stimulant effects are mediated by different neurochemical systems.

The bipolar effect of morphine on ICS was emphasized first by Lorens and coworkers [1,24]. Their study confirmed the depressant effect and showed that it was correlated with muscular rigidity. Stimulant effects also were observed and they were shown to be dependent upon dose and elapsed time after the injection. With repeated daily injections, tolerance developed to the suppressive effect and there was a concomitant enhancement of the facilitatory effect [1, 2, 9]. The latency to the onset of enhancement of ICS is comparable to the onset of morphine-induced stereotyped behavior [3,18]. Thus the sequence of suppression and enhancement of ICS may reflect the excitant and depressant effects of opiates that have been well established in other behavioral situations [34].

In a classical study on the physiological bases of morphine addiction [34] the hypothesis was advanced that the biphasic effects of opiates on behavior reflect their action on different systems in the brain. This hypothesis has received some support from studies on the locus specific effects of morphine on ICS, as cited above. More direct support was provided by a recent study on the effects of localized intracerebral injections of morphine on ICS [7,8]. In this experiment, depression without excitation was obtained after microinjections into the periaqueductal gray matter and dorsal pontine area, whereas excitation without depression was obtained with cannula implants into the hypothalamus, ventral tegmental area or substantia nigra. These results confirm that different areas of the brain mediate the bipolar effects of morphine on ICS, but the specific structures responsible for these effects still remain to be identified.

The following experiments were designed to provide a more precise description of the site that mediates the stimulant effects of opioids on lever pressing for brain-stimulation reward. In the first study injections of 1 μg of morphine were made via cannulae implanted into and around the posterior nucleus of the hypothalamus, ventral tegmental area and

[1]Please address correspondence to: C. Broekkamp, present address: Synthelabo—L.E.R.S., 31, Av. P .V. Couturier, 92220 Bagneux, France.

substantia nigra. Subsequent experiments confirmed that stimulant effects accompanied injections of lower doses of morphine (200 ng) or the metabolically stable enkephalin analog D-Ala^2-Met^5-enkephalinamide (D-ALA; [30]) into the ventral tegmental area.

EXPERIMENT 1

The results of a recent experiment [7] suggest that the excitatory effects of morphine on hypothalamic self-stimulation are mediated by neural systems originating in the ventral diencephalic-mesencephalic region of the brain. Pure excitatory effects were observed with bilateral microinjections of morphine HCl at doses from 1–2.5 μg per injection. This experiment was designed to replicate and extend these earlier findings by injecting lower doses of morphine (in 0.05–1.0 μg) into the region of the ventral tegmental area, substantia nigra and "caudal" hypothalamus. With this latter term we refer to the area of posterior nucleus of the hypothalamus and other hypothalamic tissue and nuclei at the level of the posterior nucleus.

METHOD

Animals

Male albino rats (TNO, Rijswijk, 200–250 g) were anaesthetized (50 mg/kg pentobarbital) and were positioned in a stereotaxic instrument according to the atlas of König and Klippel [19]. One electrode (Plastic Products Co. MS 303/2) and two stainless steel cannulae (0.8 mm outer dia.) were implanted chronically into the following areas of the brain. Electrode tips were implanted 5.5 mm anterior, 1.5 mm lateral and 1.8 mm above the zero point, which is the point of intersection between the interaural line and the midsagittal plane. With individual variations the cannulae were aimed bilaterally and symmetrically within the caudal hypothalamus and in the region of the ventral tegmental area and substantia nigra. The cannulae were kept patent by inserting a removable 30 gauge stainless steel stylet, which penetrated 0.5 mm beyond the tip of the outer cannula. Training for ICS commenced one week after surgery. Upon completion of the experiment, all electrode and cannulae placements were confirmed histologically.

Procedure

All experimental testing was performed during the light phase of the diurnal cycle. The test chamber measured 20×35×35 cm and a metal lever (2×4 cm) protruded from the long side of the box. Depression of the lever delivered a train of 0.2 msec positive square wave pulses, at a frequency of 100 Hz, to the electrode for a duration of 0.35 sec. Current intensities ranged between 50–250 μA. The rats were trained for 2 weeks until they displayed reliable submaximal lever pressing rates in the range of 20–50 responses/minute.

Rats displaying reliable self-stimulation behavior were adjusted to the following test schedule. A 15 min baseline stimulation test was run between 9:00–10:30 a.m. Forty-five minutes later the animals were scheduled to receive local microinjections of either 1 μg of morphine HCl bilaterally, or vehicle. Following the injection, ICS rates were obtained in a 15 min session every hour for up to 6 hr. During the intersession intervals, the animals were placed into their home cage with food and water available.

The local microinjections were made with a 31 gauge needle connected to a 5 μl Hamilton syringe. The tip of the injection needle was lowered to the same depth as the tip of the inner stylet. The stylet was removed before and replaced immediately after the injection. An injection volume of 5 μl was delivered via each cannula at a rate of 0.5 μl/30 sec. Sterile NaCl (0.9%) was used as the vehicle to dissolve and inject morphine HCl. The dose injected at the various sites was 1 μg on each side of the brain (i.e., 2×1 μg). Each experimental animal received only one bilateral injection of morphine. Control animals had bilateral implants into the ventral tegmental area or substantia nigra and were injected with vehicle only.

Following the mapping study described above, the control animals were given additional testing with lower doses of morphine. This permitted the determination of the threshold dose for morphine-induced facilitation of ICS. During this phase of the experiment, the ICS test session lasted 140 min. The animals received 3 days training on this schedule before the drug experiment commenced. The first 45 min of the session provided the baseline rate of ICS. Each animal was then removed from the apparatus and injected bilaterally with 50 or 200 ng of morphine HCl or the saline vehicle. The rat was returned to the test chamber immediately after the injection and the ICS rate was recorded for the next 90 min.

The treatment sequence was balanced across animals and the cannulae were flushed with 1 μl NaCl on the day following the first morphine injection. The two morphine injections were spaced at least 3 days apart. Each rat received more than one injection in this study because ongoing experiments had indicated that no decrement in morphine-induced facilitation occurred after five consecutive injections (H. Strijtveen, unpublished observations).

RESULTS

Mapping Effective Injection Sites

The positions of the cannulae tips for all animals (N=20) with bilaterally symmetrical placements are shown in Fig. 1. Data from two animals were discarded because of asymmetrical placements and the data from a third animal could not be used because of a lesion at the cannula tip (not in Fig. 1). All electrode tips were located in the hypothalamus. With reference to the atlas [19], the mean and standard deviation of the combined electrode loci was A. 4.6 ± 0.3 mm; L. 1.0 ± 0.2 mm, D=−2.6 ± 0.3 mm.

The cannula placements were located primarily in either the 'caudal' hypothalamus or the region of the ventral tegmental area and substantia nigra. Within the total area encompassed by the cannula placements, there were clear individual differences with respect to both the latency and intensity of the morphine-induced facilitation of ICS. At sites in the ventral tegmental area or substantia nigra, the facilitation could be observed in the first post-injection self-stimulation session (i.e., 15–30 min after morphine injection). No similar short latency effects were observed with placements dorsal to the ventral tegmental-substantia nigra, or in the caudal hypothalamus. The correlation between the distance from an optimal point within the ventral tegmental substantia nigra area (cross in Fig. 1) and the latency to the first session with the facilitation of ICS to a level greater than 150% of baseline rate was significant. A linear correlation yielded a product-moment coefficient of +0.83 ($p \leq 0.0001$).

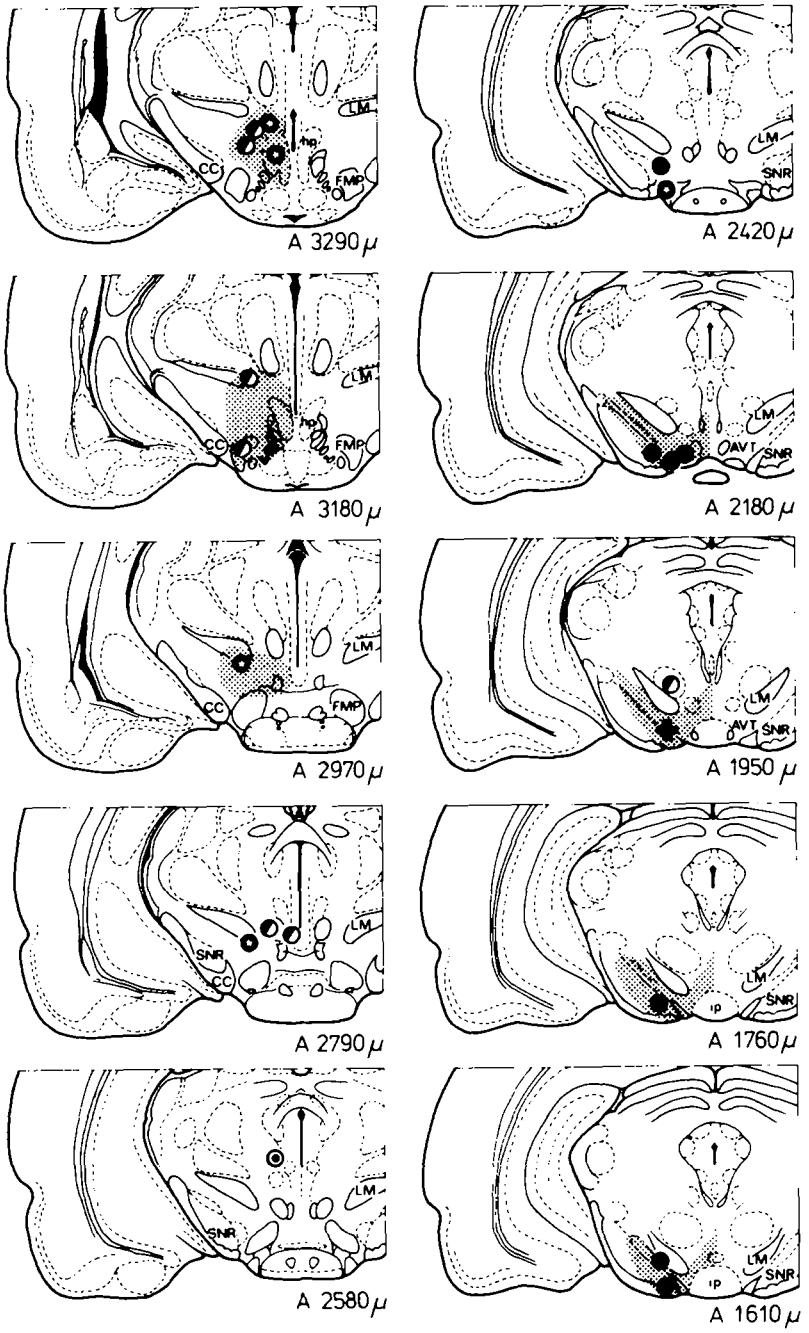

FIG. 1. Sites of injection of 2×1 μg morphine in different rats with different latencies for enhancement of self-stimulation. The solid circle indicates a cannula placement where an enhancement to at least 150% of the baseline rate was seen in the first post-injection measurement period (15–30 min after the injection). The star is used to indicate enhancements in the second post-injection period (75–90), half-solid circle for the third (135–150) and circled dot for the fourth period (195–210). The cross indicates the point from which the distance was calculated for the evaluation of the correlation between the distance from the VT-SN area and latency of the enhancement. The injection sites within the shaded areas are from those rats whose data were pooled to obtain the values represented in Fig. 2.

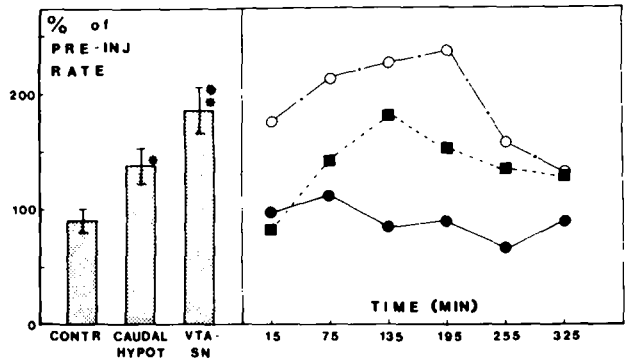

FIG. 2. Effects on self-stimulation of 2×1 μg morphine into the caudal hypothalamic area and into the ventral tegmental-substantia nigra area (VTA-SN). Bars on the left indicate the overall effect over the entire test session. Graphs on the right show the effect over time. Standard errors of the mean are presented for the overall data as well as statistical significance (**: $p \leq 0.01$; *: $p \leq 0.05$) for the difference from the control values. Baseline rates of the groups were 32, 30 and 34 resp/min for the controls, caudal. Hyp. and VTA-SN groups, respectively. ●——●: controls. ■----■: caudal hypothalamus. ○——○ VTA-SN.

A further indication of the sensitivity of the ventral tegmental-substantia nigra to the effects of local injections of morphine, comes from comparing the magnitude of response facilitation across groups. In Fig. 2 the pooled data from rats with ventral tegmental-substantia nigra placements (stippled area on plates A3290-A2970 μ, Fig. 1) are presented in comparison with results from rats in the control group and those with placements in and around the posterior hypothalamus (stippled area on plates A2180-A1610 μ, referred to as caudal hypothalamic area; Fig. 1). An analysis of variance confirmed a significant treatment effect ($F=10.4$, $p \leq 0.01$). Post-hoc Newman-Keuls tests indicated that the difference between the control group and the ventral tegmental-substantia nigra group is significant ($p \leq 0.001$) as is the difference between the control group and the caudal hypothalamic group ($p \leq 0.05$). In addition scores from the caudal hypothalamic group differ from the VT-SN group ($p \leq 0.05$).

Effect of Low Doses of Morphine

The ICS rates obtained in individual 15 min blocks after treatment with NaCl, 2×50 ng Morphine, or 2×200 ng morphine are shown in Table 1. The effect of 2×200 ng was statistically significant as revealed by analysis of variance and post-hoc tests. The slight facilitation produced by 2×50 ng of morphine was not significantly different from control scores and thus may represent the threshold value for the stimulant effect of morphine when administered by this route.

Histological examination of the brain slices confirmed that the cannulae tips were located bilaterally in either the ventral tegmental area or substantia nigra. The mean coordinates of these placements according to König and Klippel [19] were A 2.2 ± 0.3; L 1.5 ± 0.4, D −2.7 ± 0.3 mm. The electrode placements again were located in the hypothalamus (mean=A 4.6 ± 0.2; L 1.0 ± 0.2, D −2.2 ± 0.2 mm).

EXPERIMENT 2

Both the inhibitory and facilitative effects of systemically administered morphine on ICS appear to be opiate specific as they are reversed by naloxone [15]. The following experiment was designed to determine whether the excitatory effect of local injections of morphine into the ventral tegmental area also can be blocked by naloxone.

METHOD

Animals

Male albino Wistar rats (Woodlyn Farms, Ontario), weighing 300–330 g at the time of surgery, received chronic bilateral cannulae implants into the ventral tegmental area, and a unilateral electrode implant into the hypothalamus. Standard stereotaxic procedure was followed and the coordinates for the cannula placements were A 5.5, L 1.4, D −3.7 mm (König and Klippel system). Cannulae and electrode placements were confirmed histologically at the end of the experiment.

Procedure

Tests for ICS were conducted in five 22×30×40 cm Plexiglas boxes. Brain stimulation (0.2 sec brain, 60 Hz sine wave) was activated by pressing a metal lever (2.5×3.0 cm). Current intensities ranged between 8–30 μA, thus ensuring submaximal rates of lever pressing. Test sessions consisted of two phases; a 45 min baseline period, and a 90 min period following drug injections. Immediately following the 45 min baseline, the animals were removed from the test chamber and received an IP injection of either naloxone HCl (2.4 mg/kg) or the NaCl (0.9%) vehicle. The order of these injec-

TABLE 1

EFFECT OF LOCAL INJECTIONS OF 2 × 50 ng AND 2 × 200 ng OF MORPHINE HCl ON SELF-STIMULATION IN THE HYPOTHALAMUS

Injection	Overall Post-Injection rate as % of baseline	ICS rates in individual post-injection periods					
		0+15 min	15+30 min	30–45 min	45–60 min	60–75 min	75–90 min
2 × NaCl (0.9%)	96 ± 9	113 ± 19	93 ± 9	98 ± 10	90 ± 12	82 ± 10	70 ± 13
2 × 50 ng	129 ± 21	122 ± 27	159 ± 28	163 ± 42	143 ± 39	79 ± 39	104 ± 14
2 × 200 ng	219 ± 24	138 ± 33	249 ± 46	188 ± 41	247 ± 54	252 ± 57	260 ± 54

Baseline ICS rates for each treatment were: Controls: 32/min, 2 × 50 ng: 33/min, 2 × 200 ng: 28/min. The values in the table represent mean percentage of baseline rate ± SEM.

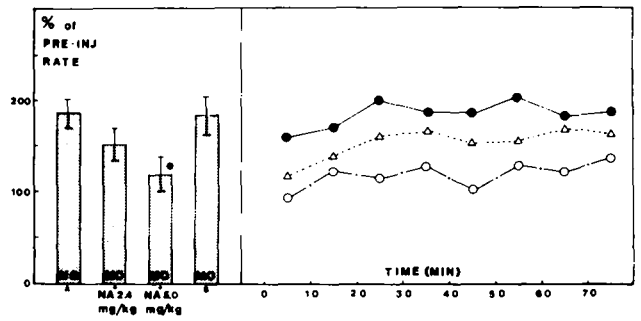

FIG. 3. Naloxone antagonism of the morphine enhancement effect on self-stimulation. Bars on the left represent the average post-injection rate over the whole 80 min post-injection period (*: $p \leq 0.05$). MORPHINE B is the morphine effect at the conclusion of the experiment. Graphs on the right show the effect over time. Baseline rates for the different measurements are 44, 45, 49 and 46 resp/min for the 2×200 ng morphine, Morph. + 2.4 nal., morph. + 5 nal and the final 2×200 ng morphine treatment, respectively. ●——●: morphine only. △---△: morphine+naloxone 2.4 mg/kg. ○—·—○: morphine+naloxone 5 mg/kg.

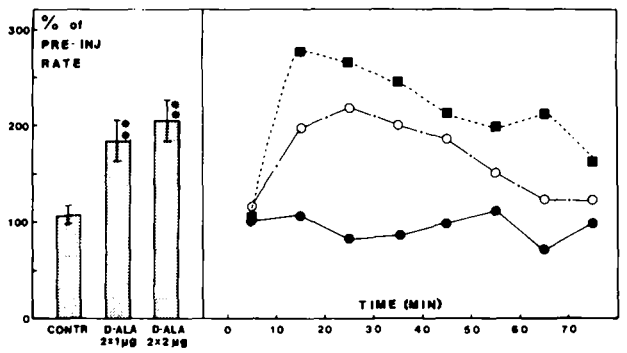

FIG. 4. D-Ala²-Met⁵-enkephalinamide on hypothalamic self-stimulation. Bars on the left represent the overall post-injection rate over the entire 80 minute test period (mean ± SEM). Data on the right show the effect over time. Absolute baseline rates were 34, 36 and 34 resp/min for the control, 2×1 μg D-ALA and 2×2 μg D-ALA measurements, respectively. ●——●: controls. ○——○: D-ALA 2×1 μg. ■----■: D-ALA 2×2 μg.

tions was counterbalanced within the group. After the IP injections, all animals received bilateral intracranial injection of morphine (2×200 ng) in accordance with the procedure described in Experiment 1. The experiment was repeated with a 5 mg/kg dose of naloxone. To confirm that the effect of morphine does not change over repeated injections, the study concluded with a final bilateral treatment with morphine. The experimental injections were spaced at least 2 days apart and 1 μl of sterile NaCl (0.9%) was flushed into the cannulae on each day following a morphine injection.

RESULTS

The data employed in the statistical analysis came from the animals with symmetrical cannula placements in the ventral tegmental area, which completed all phases of the experiment (N=6). The mean coordinates for the cannulae were A 2.2 ± 0.2, L 0.9 ± 0.2, D −2.7 ± 0.3 mm. All electrodes were located in the hypothalamus mean ± SD=A 4.3 ± 0.3, L 1.0 ± 0.2, D −2.5 ± 0.3.

The effects of the two doses of naloxone on the facilitory effect of morphine on hypothalamic ICS are shown in Fig. 3. The higher dose of naloxone (5 mg/kg) effectively antagonized the stimulant effect of morphine but the lower dose of 2.4 mg/kg was only partially effective. A linear trend analysis confirmed a significant reduction in ICS after 5 mg/kg naloxone as compared to the first morphine injection ($p \leq 0.05$). A comparison of the first and last morphine injections indicates that the effectiveness of morphine was not attenuated after repeated testing.

EXPERIMENT 3

The data from Experiments 1 and 2 indicate that the direct stimulation of opiate receptors in the region of the ventral tegmental area produced a facilitation of ICS in the hypothalamus. As a further test of this hypothesis the following experiment sought to determine whether similar effects could be produced by microinjections of the metabolically stable enkephalin analog [30] D-Ala²-Met⁵-enkephalinamide (D-ALA) into the ventral tegmental area.

Animals

Male albino Wistar rats were prepared with cannulae and electrodes as described in Experiment 2.

Procedure

Tests for ICS were similar to those described above, as was the procedure for local microinjections. D-ALA (Calbiochem) was dissolved in sterile NaCl (0.9%) in plastic tubes. Two doses of the peptide were used (i.e., 2×2 μg; 2×1 μg). As in Experiment 2, testing for ICS resumed immediately following the microinjections and continued for 90 min.

RESULTS

Only those data from animals with symmetrical cannulae placements (A 1.7 ± 0.4, L 0.9 ± 0.2, D −2.8 ± 0.3) were retained for statistical analysis (N=13). Electrode placements were confirmed to be in the hypothalamus (A 3.5 ± 0.4, L 1.3 ± 0.2, D −3.0 ± 0.3).

As may be seen in Fig. 4, both doses of D-ALA (2×1 μg, 2×2 μg) produced a strong facilitation of hypothalamic ICS. A comparison of the overall rates for each of the three treatment conditions revealed a significant effect with both doses of D-ALA ($p \leq 0.05$). The duration of the effect of 2×1 μg D-ALA was approximately 70 min and peak facilitation was reached after 20 min. Thus the duration of the enkephalin effect was shorter than that seen after local injections of morphine.

GENERAL DISCUSSION

The present results extend the findings of an earlier study [7] by showing that microinjections of opioids into the substantia nigra and ventral tegmental area led to an enhancement of hypothalamic ICS. The relatively short latency and the magnitude of enhancement indicate that the effects of morphine may be mediated by neurons in a region encompassing the substantia nigra, ventral tegmental area and closely adjacent interpeduncular nucleus (IP-VT-SN area).

A weaker response and a longer latency was observed after injections into the caudal area of the hypothalamus. This observation could be accounted for by diffusion towards the IP-VT-SN area.

The sensitivity of the area under discussion is substantiated further by the fact that a significant enhancement occurred with a dose of morphine as low as 2×200 ng. The latency of at least 10 min between injection and clear facilitation of ICS is somewhat perplexing. However, this may be due to the amount of diffusion necessary to recruit a sufficiently large population of neurons for enhancement to occur. Diffusion to some nucleus closely adjacent to the cannula tip, e.g., the interpeduncular nucleus is another possibility. In fact, the problems associated with diffusion which accompany local microinjections, preclude a more precise description of the exact site of action within the IP-VT-SN area.

These behavioral effects can be related to opiate receptors that have been shown to be present in the area in moderate density [2,32]. Also, immunofluorescence studies have identified both cell-bodies and terminals containing enkephalin or enkephalin-like material within the IP-VT-SN area [35,36]. The observation that naloxone is able to antagonize the morphine effect shows that genuine opiate receptors, as defined by naloxone binding and antagonism, mediate the presently described effects. The possibility that the antagonism by naloxone is due to an inhibition of self-stimulation per se can be discounted. In a previous study [36] no attenuation of hypothalamic ICS was observed after naloxone injections at doses used in the present experiment. The similar increase in ICS rates following microinjections of D-ALA into the VT area provides further support for the hypothesis that these effects are mediated by opiate receptors. There are data obtained with isolated organs which suggest the existence of different opiate receptors labeled as μ, δ and κ-receptors [36]. The δ receptor has an inferior sensitivity with respect to naloxone antagonism. It is premature to speculate on the type of receptor involved in the presently localized central effect but the relatively high dosage of naloxone that is needed to antagonize the morphine induced enhancement is relevant for this issue.

On the basis of the present data and those of an earlier microinjection study [7], it is suggested that the stimulant effects of opioids, whether administered systemically or centrally are mediated by their action within the IP-VT-SN area. In fact, several lines of evidence can be summarized in support of the hypothesis that the opiate induced stimulant effect in general, be it hyperexcited behavior in cats [12,13], locomotor activity in mice [10] or stereotyped behavior in rats [3] is initiated in this area. Thus, it is shown [16] that stereotyped behavior can be elicited by injections of β-endorphin into the substantia nigra. In previous studies we have found that locomotor stimulant effects can be elicited by injections of D-ALA into the VT area of rats [6]. Locomotor stimulant effects also occur after low doses of systemically applied morphine [4,5]. Finally, with naloxone infusions into the substantia nigra of cats it is found that the hyperactive behavior induced by IP morphine is antagonized and a rigid state of immobility remains [37]. Thus, although some of these data were obtained with different drugs, these drugs all have in common the fact that they can interact with opiate receptors, including those located within the IP-VT-SN area.

There is now convincing evidence that the facilitative effects of psychomotor stimulants on ICS are mediated by dopamine pathways [11,31] which originate in the same region of the brain [21] where opioids produce their maximal effect on ICS. These correlational data are consistent with several other lines of evidence which suggest that the stimulant effects of opioids may involve dopamine neurons. For example, disruption of catecholamine biosynthesis with α-methyl-para-tyrosine blocks morphine-induced facilitation of ICS rate [29]. Neurophysiological data are also consistent with this suggestion as it is reported [17,26] that single-unit firing rates of dopamine neurons are increased significantly following systemic injections of morphine. Furthermore, neuroleptic drugs block the excitatory effects of morphine in cats [12,13], mice [10] and rats [3]. Future studies combining selective neurotoxic lesions of ascending dopamine systems, with localized injections may help to determine whether the enhancing effects of opioids on ICS are mediated by dopaminergic neurons.

As motor excitation and enhanced self-stimulation can be initiated within the same area by application of opiates [6] it remains to be studied whether the effects on ICS are indirect. With systemically injected morphine there is now a considerable body of evidence showing facilitation of ICS in paradigms which control for locomotor activity through the use of rate-independent measures such as threshold intensity determination [14, 15, 25]. One property that the threshold technique has in common with rate measures of ICS is the lack of tolerance to the enhancement effects [14,24]. An important goal for future experiments will be to determine whether reduction in ICS thresholds and the absence of tolerance can be demonstrated with local injections of opioids into the presently identified morphine sensitive area of the mesencephalon.

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