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Locus Coeruleus and Substantia Nigra: Involvement in Morphine-Induced Behavior

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VAN DONGEN, P. A. M., C. L. E. BROEKKAMP AND A. R. COOLS. *Locus coeruleus and substantia nigra: involvement in morphine-induced behavior.* BRAIN RES. BULL. 4(3) 307–311, 1979.—Cats pretreated with morphine (5 mg/kg, IP) received naloxone into the area of the locus coeruleus (LC) or the area of the substantia nigra (SN). The LC-treated animals stopped the morphine-induced stereotyped behavior and showed normal but hyperactive behavior. The SN-treated animals, however, ceased their movements of the head and the forelegs, adopted a rigid posture with extended forelegs and became hypoactive. It is concluded that both the LC area, which contains noradrenergic cell bodies, and the SN area, which contains dopaminergic cell bodies, are sites of action of morphine on behavior.

Locus coeruleus Substantia nigra Norepinephrine Dopamine Morphine Naloxone Behavior

NUMEROUS investigations deal with the interaction between morphine, enkephalins, enderphins and the central catecholaminergic transmission. In rats, for instance, morphine-induced analgesia, behavioral activation and abstinence appear to be influenced by drugs which affect catecholaminergic transmission [5, 6, 22, 23] or by destruction of catecholaminergic structures [19,21]. In cats, manipulations of dopaminergic (DA) and noradrenergic (NE) transmission influence morphine-induced behavioral symptoms [7, 8, 9, 10]. Although both noradrenergic and dopaminergic terminal structures contain morphine-sensitive receptors, local administration of morphine in the terminal areas investigated does not affect open field behavior [8,9]. Morphine intracerebrally injected into the pontine area of NE-cells, the locus coeruleus (LC), decreases self-stimulation behavior [4]. Moreover, morphine intracerebrally injected into the mesencephalic area of DA-cells, the substantia nigra (SN), causes an increase in self-stimulation behavior and in dyskinetic leg movements [3,4]. The present study attempts to elucidate whether morphine-receptors in the areas of the LC and SN play a role in the behavior elicited by systemic administration of morphine. As morphine-receptors can be antagonized by naloxone, the effects of naloxone locally injected either into the LC or into the SN on morphine-induced behavior are investigated.

METHOD

The methods have been extensively described elsewhere [7, 8, 26]. Under pentobarbital (30 mg/kg, IP, Nembutal®) or halothane (0.4–0.8% in O_2/N_2O; 1/2) anaesthesia, two cannulas directed to the LC (Horsley-Clarke coordinates P 2.5; L 2.0, H −2.0) or two cannulas directed to the SN (Horsley-Clarke coordinates A 5.5, L 3.5, H −5.0) were implanted in 30 adult cats of both sexes. After a 14 day recovery from the operation, the animals received an intraperitoneal injection of morphine (5 mg/kg) and their behavior was observed via a closed TV-circuit. Forty min after the injection of morphine, the animals received a micro-injection of naloxone (0.8, 2.0, 5.0 or 10.0 μg in 0.5 μl saline) or its solvent in the area of the LC or the SN. The most conspicuous changes after the injection of naloxone were quantified by counting the affected behavioral categories: head movements, leg movements and stereotyped turnings, i.e. turnings about the hindlimbs or circling throughout the cage which are repeated in a rigid way. For statistical analyses, the number of movements and of stereotyped turnings were counted per minute during 10 min immediately before and 10 min immediately after the intracerebral injections. This resulted in two time series for each cat, which were, in addition, averaged for each test-group. Both comparisons of the post-injection time series with the preinjection time series (unpaired, Mann-Whitney U) and comparisons of the postinjection time series of control groups with those of the treated groups (time-paired, Wilcoxon) were made. Intertrial-interval varied from 2 days (SN experiments) to 7 days (LC experiments), when the animals received more than one injection of morphine. Except for an increase in salivation and a decrease in defecation and vomiting, no signs of tolerance or abstinence could be demonstrated for this dose and schedule of injections. After the experiments, the cats received an overdose of pentobarbital, were transcardially perfused and the injection sites were localized as described previously [26]. The areas of catecholamine (CA)-containing cell bodies were demarcated according to Maeda *et al.* [14] and Poitras and Parent [18]. The area of the NE-cells extends from the LC to structures ventrolateral to this nucleus around the brachium conjunctivum, and overlaps the nucleus pontis centralis caudalis. Other structures were demarcated according to

Berman [1] and Taber [25]. Since it may be assumed that the drug spreads about one mm from the injection site, sites inside an anatomically demarcated structure and at a distance of less than one mm are lumped together. The following drugs were used: naloxone-HCl (gift of Endo Laboratories) and morphine-HCl ("De onderlinge pharmaceutische groothandel"). All doses refer to the salts.

RESULTS

Injections in the Area of the LC

Sites of injection aimed at the LC were located in the NE-area, the nuclei pontis centrales oralis and caudalis, the nucleus laterodorsalis tegmenti, the central gray and in and adjacent to the fourth ventricle. As brain damage around the site of injection was observed in one cat, the data of this animal were discarded.

General Behavioral Observations

Morphine-induced behavior. After the intraperitoneal injection of morphine, the animals showed the behavioral sequences characteristic for morphine-treated cats (cf. [7, 8, 28]). Thirty to sixty min after the injection of morphine, the cats showed morphine-induced repetitive movements [7]. These movements were poorly coordinated and consisted of repeated sequences of disintegrated behavior. Morphine-treated cats were hyperactive, as expressed by the high frequency of head and body movements [8]. Between 30 and 40 minutes, the mean number of stereotyped rotations per min was 2.83 (n=91); during this time a small, but not significant tendency for an increase in the frequency of stereotyped turnings was found (Friedman two-way analysis of variance, $\chi^2_R=7.5$, $df=7$, $0.30<p<0.50$). The reaction to different sensory stimuli was inadequate: cats often appeared to track objects visually which were not present, or they bumped against the walls; they either reacted not at all to auditory stimuli or they showed an exaggerated startle response instead of an orientation reaction. Moreover, the cats often dirtied their fur with feces or vomit when morphine caused defecation or vomiting.

Saline. Saline itself (0.5 μl, n=9) produced an increase in the number of stereotyped turnings (Fig. 1A, compared with pre-injection level $p<0.01$, Mann-Whitney U). A comparison of pre-injection with post-injection levels per animal resulted in a statistically significant increase in 3 out of 9 tests: these animals showed an intensified morphine-induced behavior. (A comparable increase in activity was also noted after injections of saline in the area of the SN, Fig. 1B).

Naloxone. Within a few minutes after an unilateral or bilateral microinjection of naloxone into the dorsolateral pontine tegmentum the following effect was found, depending on dose and injection site (see below): the cats stopped their morphine-induced behavior (Fig. 1A). The number of morphine-induced stereotyped turnings decreased significantly both compared to pre-injection levels and to saline treated controls (Fig. 1A). Instead of the morphine-induced stereotyped movements, the cats walked, sniffed, explored, rolled, sharpened their nails, groomed, scratched their heads, stretched, made smoother head movements, did not show the exaggerated startle response nor dirtied their fur with feces or vomit any more. The cats remained hyperactive: no change in the number of head and leg movements was observed and normal inactive behavior like lying or sleeping was absent till the end of the observation. After an effective injection, the decrease in the morphine-induced behavior in each individual cat was greater than suggested by the mean values (Fig. 1A), since at every dose of naloxone ineffective injections occurred (see below): in fact, the stereotyped movements per animal disappeared completely or almost completely for 2 to 20 minutes. Morphine-induced effects which remained unaffected following an otherwise effective injection of naloxone near the LC were: mydriasis, restless movements of the forelegs, salivation, the crouched back, and the posture of the hindlimbs characteristic for defecation (i.e. flexion in the hip and knee and extension in the heel). The naloxone-induced effect faded away after several minutes (Table 2) and the previously shown stereotyped patterns reappeared. The intensity of the naloxone-induced partial antagonism of the morphine-induced behavior varied with the individual cats ranging from smoother, more oriented head movements to a decrease in stereotyped movements and normalized active behavior.

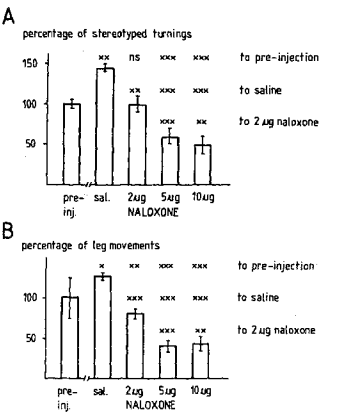

FIG. 1. Effects of naloxone injected in the area of the LC and the SN on morphine-induced stereotyped turnings and leg movements. A. Number of stereotyped turning (mean and standard error of the mean) following injections in the dorsolateral pontine tegmentum. B. Number of leg movements (mean and standard error of the mean) following injections in the SN area. Comparison with pre-injection level: Mann-Whitney U; comparison with saline-treated controls and with 2 µg naloxone: Wilcoxon. (The pre-injection levels are the mean values of all the LC- and SN-experiments respectively.) $^{\times}p<0.05$; $^{\times\times}p<0.01$; $^{\times\times\times}p<0.001$

TABLE 1

DISTRIBUTION OF EFFECTIVE AND INEFFECTIVE SITES OF INJECTION FOR NALOXONE-INDUCED PARTIAL ANTAGONISM OF THE MORPHINE-INDUCED BEHAVIOR AFTER AN UNILATERAL INJECTION OF 2 μG OF NALOXONE. SINCE SITES AT A DISTANCE OF LESS THAN 1 MM ARE INCLUDED (SEE METHODS) THE SUM OF SITES IN DIFFERENT AREAS EXCEEDS THE TOTAL NUMBER

In and near the areas	Number of effective sites (N_e)	Number of ineffective sites (N_i)	$\frac{N_e}{N_e+N_i}$ (%)	Ration of N_e and total number of effective sites (%)
fourth ventricle	3	7	30	21
NE-area	12	19	39	86
n. pontis centralis caudalis (PCC)	8*	9	47*	57*
n. pontis centralis oralis (PCO)	6*	18	25*	43*
n. laterodorsalis tegmenti	1	11	9	7
Total	14	34	29	100

*effective sites in the PCC and PCO were mainly found in and near the NE-area (PCC: 7 out of 8; PCO: 5 out of 6)

TABLE 2

LATENCY AND DURATION OF NALOXONE-INDUCED BEHAVIORAL EFFECTS. INDICATED ARE THE MEAN VALUES AND BETWEEN BRACKETS THE RANGE

	Injections in the LC Partial normalization of the behavior		Injections in the SN Behavioral inactivation	
Dose	Latency	Duration	Latency	Duration
2.0 μg	1.2 (0–7.0)	5.0 (2.6–8.0)	1.7 (0–5.0)	>15
5.0 μg	0 (0)	6.3 (2.5–8.8)	1.0 (0–5.0)	>15
10.0 μg	1.6 (0–4.2)	21.2* (14.3–31.3)	3.0 (0–15.0)	>15

*$p<0.001$, Mann-Whitney U.

In conclusion, naloxone partially antagonized the morphine-induced disturbance of behavior.

Dose-Response Relationship

The naloxone-induced effect was reproducible per cat: a second injection in a previously effective site was always effective (n=5), while a second injection in an ineffective site never was (n=10). The naloxone-induced effect was dose-dependent: at doses of 5 μg (n=6) and 10 μg (n=13) naloxone, the intensity (Fig. 1A) and the duration (Table 2) increased significantly compared with the dose of 2 μg (intensity: $p<0.001$ and $p<0.01$ for 5 and 10 μg, respectively, Wilcoxon; duration: $p<0.001$ for 10 μg, Mann-Whitney U); moreover, more sites of injection became effective. Bilateral injections were more effective: at a dose of 0.8 μg (2x) a statistically significant decrease in the number of stereotyped turnings was observed in 40% of the cases (n=15), while only 29% of the cases were effective after unilateral injections of 2 μg (n=48). Bilateral injections of 0.8 μg caused a decrease of the number of stereotyped turnings to 33% of the number found in the saline-treated controls (compared with pre-injection levels $p<0.05$, Mann-Whitney U; compared with saline-treated controls $p<0.001$, Wilcoxon), while an unilateral injection of 2 μg caused only a decrease to 71%. No effect on the behavior was caused by naloxone (2 μg) injected in non-morphinized cats (n=13).

Localization

The distribution of the effective injection sites is indicated in Table 1. In and near the NE-area 12 out of the 14 effective sites were found. In addition the nucleus centralis caudalis (PCC), which partly overlaps the NE-area, contains 8 effective sites. In contrast, the nucleus laterodorsalis contains the smallest amount of effective sites.

Injections in the Area of the SN

General behavioral observations. Naloxone bilaterally injected (2×2 μg) at effective sites in the area of the SN of morphine-treated cats (n=7) made these animals hypoactive. The number of stereotyped movements, head movements, and leg movements decreased significantly compared with preinjection levels ($p<0.05$, Mann-Whitney U) and with saline-treated controls ($p<0.001$, Wilcoxon, Fig. 1B). After effective injections of naloxone, the animals sat with the upper part of the body upright and the forelegs extended. The morphine-induced crouched back, the hindlimbs in the posture characteristic for defecation, mydriasis and salivation remained unaffected by otherwise effective injections of naloxone. This effect was dose-dependent (Fig. 1B). At a dose of 2 μg, in 3 out of 7 cats a decrease in the activity was observed, at a dose of 10 μg, 5 out of 7 cats became hypoactive. Naloxone injected near the SN of morphine-treated cats made these animals hypoactive, in contrast to microinjections near the LC, after which the animals remained hyperactive. So, the effects elicited from both areas were essentially different, although the number of stereotyped movements decreased after effective injections of naloxone in both areas.

Localization

The effective sites of injection were located in the ventromedial part of the SN pars compacta, in the ventral tegmental area (VTA) and just ventral to the SN. Of the 2 inef-

fective sites of injection, one was located in the SN pars compacta and one rostrodorsally in the tegmental fields of Forel.

DISCUSSION

The Area of the Pontine NE-Cells

Naloxone injected into the dorsolateral pontine tegmentum of morphine-treated cats partially antagonized the morphine-induced disturbance of the behavior in a number of cats. Although only a limited number of sites localized within the same anatomically demarcated area was effective (Table 1), the effect was reproducible per site and a clear cluster of effective sites was found. The cells involved in the naloxone-induced partial normalization of the behavior may be the NE-cells or the non-catecholaminergic cells of the nucleus pontis centralis caudalis (PCC), intermingled with the former cells and situated ventrally to the LC and the subcoeruleus area (SC). The present study permits no definite conclusion regarding the group of cells involved but 86% of the effective sites were located in and near the NE-area (Table 1). This clearly suggests that this area is the most effective structure. So, it can be tentatively concluded that the NE-cells mediate the effect observed. This conclusion is strengthened by the following data: (a) opiate receptor binding is very high in the LC and much lower in the more ventral PCC [17], and (b) in the dorsolateral pontine tegmentum, the morphine-sensitive cells are mainly confined to the LC [2,29]. The technique used, however, does not allow us to decide whether the naloxone-induced effect is due to actions of naloxone on the SC cells or the dorsal or ventral part of the LC.

In rats, morphine causes a decrease in the ongoing activity of the LC-cells [2,29], which can be antagonized by naloxone. Accordingly, the naloxone-induced effects can be ascribed to the restoration of the activity of the NE-cells in morphine-treated cats and thereby to the restoration of the activity of NE-terminals over almost the whole central nervous system. This is in agreement with the observation that an injection of NE into a single LC terminal area also interrupts the morphine-induced stereotyped movements [8]. As in this study all NE-terminal areas were influenced, it is not surprising that naloxone injected in the area of the pontine NE-cells caused a more generalized behavioral effect: partial normalization of the behavior.

The Area of the Mesencephalic DA-cells

Naloxone injected in the area of the mesencephalic DA-cells of morphine-treated cats ended the morphine-induced hyperactivity of these animals and, instead, made the cats hypoactive. The activity of the dopaminergic SN-cells is increased by morphine; this effect can be antagonized by naloxone [11,15]. So, it is reasonable to assume that naloxone injected in the area of the mesencephalic DA-cells diminished the DA-release in the caudate nucleus and other DA terminal areas. Indeed, the activity of morphine-treated cats is also modified by DA-agents injected in particular parts of the caudate nucleus [9]. Although the present study indicates the involvement of mesencephalic DA-cells, it does not permit conclusions regarding the involvement of the particular group of cells: the SN pars compacta (A9) or the VTA (A10).

NE- and DA-cells in Morphine-induced Behavior

These results partly parallel the effects of morphine agonists in the NE- and DA-areas on behavior of the cat and rat [3,4]. Naloxone in and near the pontine NE area of morphine-treated cats normalized the behavior, while the animals remained hyperactive; conversely, morphine agonists in and near the NE-area of rats and cats elicit hypoactivity in self-stimulation and exploration test situations respectively [4,27]. Naloxone injected in and near the mesencephalic DA-areas of morphine-treated cats makes them hypoactive and decreased the number of morphine-induced abnormal leg movements; conversely, morphine in and near the mesencephalic DA-area of rats and cats makes them hyperactive and increased the frequency of dyskinetic leg movements in the self-stimulation and open field test situation respectively [3,4].

As discussed above, the morphine-induced stereotyped behavior could be interrupted by restoring the activity of the NE-cells or by diminishing the stimulation of DA-receptors in the caudate nucleus, either by injection of DA antagonists in parts of the caudate nucleus [9] or by injection of morphine antagonists in and near the DA-cell bodies. Yet, microinjections of morphine agonists neither in the area of the pontine NE-cells [4,27] nor in the area of the mesencephalic DA-cells [3,4] nor in the caudate nucleus (cf. [9]) alone induces stereotyped behavior. On the basis of the present data, it becomes attractive to suggest that morphine-induced behavior requires a simultaneous action on morphine receptors in the areas of the pontine NE-cells and mesencephalic DA-cells.

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