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Intraseptally Injected Opiate Agents: Effects on Morphine-Induced Behaviour of Cats

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MEGENS, A. A. P. H. AND A. R. COOLS. *Intraseptally injected opiate agents: Effects on morphine-induced behaviour of cats*. PHARMAC. BIOCHEM. BEHAV. 17(2) 297–304, 1982.—Behavioural effects of intraseptally administered opiate agents were analyzed in cats pretreated with an intraperitoneal injection of morphine. In this way, it became possible to investigate (1) the involvement of septal opiate receptors in the behavioural response of cats to systemic administration of morphine, and (2) the pharmacological character of septal opiate receptors. The following results were obtained with intraseptal injections 15–16 min after intraperitoneal morphine: (1) naloxone decreased frequencies of head and limb movements, and (2) morphine was ineffective. The following results were obtained with intraseptal injections 40–41 min after intraperitoneal morphine: (1) β-endorphin and, to a lesser extent, fentanyl increased frequencies of locomotor patterns, (2) morphine and Met-enkephalin were ineffective, (3) naloxone and naltrexone decreased frequencies of locomotor patterns in a dose-dependent way, (4) naloxone and naltrexone antagonized the effects of β-endorphin and fentanyl, and (5) morphine did not attenuate the effect of naloxone. The intraseptal injections affected only the frequencies of the systemically evoked behaviour patterns; the nature of the behaviour patterns remained unchanged. It is concluded that (1) systemically administered morphine does not affect behaviour via a direct action on septal opiate receptors, and (2) the receptors mediating the septally evoked effects are most probably ε-type opiate receptors. The hypothesis is put forward that systemic administration of morphine results in an increased release of β-endorphin from hypothalamo-septal neurons and, as a consequence, changes the β-endorphin activity at the ε-type opiate receptors in the septum.

Septum Multiple opiate receptors Morphine Opioid peptides Behaviour Cat

MORPHINE and related opiate agonists produce a wide variety of effects, which are thought to be mediated via specific receptor sites within the central nervous system (for reviews see [26, 34, 44, 46]. The septum is one of the brain regions that contain both high densities of opiate receptors and high concentrations of opioid peptides [4, 24, 30, 48, 51, 56]; moreover, intraseptal injections of opiate agents are biochemically effective [6, 40, 41]. Therefore, some of the effects elicited by systemic administration of morphine may be mediated via septal opiate receptors. Although septal lesions have been reported to suppress morphine-induced analgesia [7,42], intraseptal injections of morphine or β-endorphin do not evoke analgesia [22, 23, 40, 41]. Hence, septal opiate receptors are apparently not a site of action for the analgesic effects of opiates. On the other hand, there is indirect evidence that septal opiate receptors are involved in the behavioural effects of opiates: (1) septal lesions affect the behavioural effects of morphine in rats [29]; for reviews on the role of the septum in behaviour see [17,18], and (2) septal neuronal systems play an important role in the behavioural response of cats to morphine administration [36, 37, 47]. In the present study, an investigation is carried out into whether septal opiate receptors are indeed a site of action for the behavioural effects of systemically given opiates. Consequently, we have investigated the ability of intraseptally injected opiate agonists and antagonists to potentiate or suppress the behavioural effects of systemically given morphine in cats.

The behavioural effects of systemic injections of morphine in cats have been extensively described elsewhere [11, 12, 13]. Administration of a moderate dose of morphine (5 mg/kg, IP) causes the development of 3 behavioural phases, which appear in succession: depression, re-organization and ritualization. The second phase or re-organization phase, which starts about 10–15 min after the morphine injection is characterized by the break-down of normal head, body and postural movements into an increasing number of isolated, staccato-like movements, and the subsequent appearance of new head, body and postural movements in which the single units are re-integrated; the resulting patterns, which are individual-specific, become stereotyped, i.e., continuously repeated in a specific sequence. The last phase or ritualization phase, which start about 30 min after morphine injection, is characterized by a restricted number of senseless, stereotyped behaviour patterns that are regularly repeated at a stabilized level. As septal opiate receptors may be differentially involved in these behaviourally distinct morphine-induced phases, the present study analyzes the effects of intraseptal injections of opiate agents both during the reorganization phase (i.e., injections 15–16 min after intraperi-

toneal morphine) and during the ritualization phase (i.e., injections 40–41 min after intraperitoneal morphine).

The existence of multiple opiate receptors has recently been reported [10, 27, 32, 35, 57]. In the present study, we inject 4 different opiate agonists (morphine, fentanyl, Met-enkephalin and β-endorphin) into the septum during the ritualization phase. These 4 opiate agonists have different ranking orders of potencies at 3 different types of opiate receptors [8, 9, 30, 57]: (1) the μ-receptor (fentanyl> β-endorphin>morphine>Met-enkephalin), (2) the δ-receptor (Met-enkephalin>fentanyl>β-endorphin>morphine), and (3) the ε-receptor (β-endorphin>fentanyl>>Met-enkephalin, morphine). By determining the ranking order of potencies of these 4 opiate agonists at septal opiate receptors, it may be possible to get more insight into the pharmacological character of the septal opiate receptors. The opiate antagonists used in the present study, naloxone and naltrexone, are about equally potent at μ- and ε-receptors, but somewhat less at the δ-receptor [8, 9, 31, 39, 59].

METHOD

General

Adult cats (2.0–4.0 kg) of both sexes were used. Cannulas aimed at the septum were implanted bilaterally by means of a stereotaxic procedure under pentobarbital anaesthesia (Nembutal® or Narcovet®, 50 mg/kg, IP). The double-barrelled, stainless steel cannulas were fixed on the skull with acrylic dental cement; the diameters of the guide and inner cannulas were 0.8 mm and 0.5 mm, respectively. After a 2 week recovery period, the animals showing no overt behavioural signs of brain destruction were habituated to the experimental cage and the injection procedure for two separate periods of 1 hr each. A quarter of an hour prior to each experiment, the animal was placed in the observation cage (88×66×61 cm). This observation cage was sound proof and equipped with a ventilator producing background noise. With exception of the perspex front window the walls were opaque. During the experiments care was taken to prevent the occurrence of changes in the immediate surroundings of the cage. Behaviour was recorded on videotapes by means of closed-circuit television (for additional information, see [11–13, 36, 37].

Intraseptal injections, either bilateral (2×) or unilateral (1×) were carried out manually with Hamilton injection syringes (diameter of the needle: 0.4 mm). Each injection took about 5–10 sec. The whole injection procedure was carried out within 2 min. The following agents were used: morphine hydrochloride (De Onderlinge Pharmaceutische Groothandel), fentanyl citrate (a gift from Janssen Pharmaceutica), human β-endorphin and Met-enkephalin (gifts from Organon), naloxone hydrochloride and naltrexone hydrochloride (gifts from Endo Laboratories). The injection volume was 0.5 μl for all agents with the exception of morphine, which was injected in a volume of 1.0 μl. The intracerebrally administered drugs were dissolved in distilled water (with the exception of the opioid peptides, which were dissolved in 0.01 n HCl); the intraperitoneally injected morphine was dissolved in saline (0.9% NaCl). All doses, apart from those for the opioid peptides, refer to the salts. The solvents were sterile and apyrogenic.

The intraseptal injections were given in cats pretreated with an intraperitoneal injection of morphine (5 mg/kg). When animals were used more than once, an intertrial interval of at least 2 weeks was used. Using this time interval, no signs of tolerance or abstinence were seen, apart from the observation that cats receiving a second injection of morphine showed incidentally some salivation either during the transport from the home cage to the experimental cage or during the experiment itself. However, test groups comprising non-naive animals contained always a number of naive cats; no difference in the effectiveness of the intraseptal injections was observed between naive and non-naive cats.

After the experiments, the animals were sacrificed with an overdose of pentobarbital and perfused with a formaldehyde solution (4–10%), containing the anti-coagulant heparin (50 mg/l). The brains were removed and sectioned along the tracks of the guide cannulas. The injection sites were localized with reference to Snider and Niemer's atlas [53]. For nomenclature the subdivision of the septum in the cat as proposed by Andy and Stephan [2] was used.

Behavioural Analysis

The animals received an intraperitoneal injection of morphine and were observed for a period of 60 min immediately following the morphine injection. Drugs were injected intraseptally either during the re-organization phase (15–16 min after intraperitoneal morphine) or during the ritualization phase (40–41 min after intraperitoneal morphine). The drug-induced changes in behaviour were measured both qualitatively (description of the behaviour patterns) and quantitatively (scores of frequencies of head movements, limb movements, and locomotor patterns).

Intraseptal Injections 15–16 min After Intraperitoneal Morphine

This stage of the morphine syndrome is characterized by increasing frequencies of head and limb movements of the morphine-treated cats (see Results section). Therefore, frequencies of head and limb movements were used as an experimental variable for the quantitative analysis of the septally evoked effects at this point of time. The period of 44 min immediately following the intraseptal injections was subdivided into 11 periods of 4 min each. For each animal, scores of head and limb movements per 4 min period were determined. These scores were normalized by expressing them as percentages of the total number of head and limb movements displayed by each animal during the whole post-injection period of 44 min. In this way, the ongoing development of the morphine syndrome following the intraseptal injections was represented independently of inter-individual differences in activity. For statistical analysis the percentages determined for all animals in each test group were compared per 4 min period with the corresponding percentages measured in control animals treated with distilled water (2-tailed Mann-Whitney U-test).

Intraseptal Injections 40–41 min After Intraperitoneal Morphine

This stage of the morphine syndrome is characterized by the continuous display of stereotyped behaviour patterns at a stabilized level (see Results section). Therefore, frequencies of locomotor patterns were used an an experimental variable for the quantitative analysis of the septally evoked effects at this point of time. Single locomotor patterns were defined as: (1) any forward or backward movement of the animal along a minimum distance of 40 cm in the case of discontinuous

locomotion, (2) any forward or backward movement of the animal along a fixed distance of 80 cm in the case of continuous locomotion, (3) any turning movement of the animal around a minimum angle of 180° in the case of discontinuous turning, and (4) any turning movement of the animal around a fixed-angle of 360° in the case of continuous turning. For each animal, scores of locomotor patterns were determined both over the 10 min period immediately preceding the intraseptal injections (X_{pre}) and over the 10 min period immediately following the intraseptal injections. Behaviour was not analyzed during the 2 min injection period. Further analysis of the effects was carried out in a different way for the opiate agonists and antagonists, for reasons to be mentioned below.

Opiate agonists. The absolute difference in the number of locomotor patterns scored during the pre- and post-injection periods per animal was determined by:

$$\Delta_{abs} = X_{post} - X_{pre}$$

The values obtained in this way per test group were compared with the corresponding values determined for the appropriate control group (Mann-Whitney U-test).

Opiate antagonists. In contrast to intraseptal injections of opiate agonists, intraseptal injections of opiate antagonists resulted in decreased frequencies of the morphine-induced locomotor patterns. Therefore, statistical analysis of these effects had to be performed differently from the procedure outlined above for opiate agonists. First, decreases in locomotor activity can only be determined in animals showing at least some locomotor activity during the pre-injection period. Hence, only animals with a minimum number of 10 locomotor patterns during the pre-injection period were used to analyse the effects of the opiate antagonists on locomotor activity. Furthermore, the magnitude of decreases in locomotor activity is dependent on the pre-injection values X_{pre}, which considerably differed between animals. Accordingly, the relative change in the number of locomotor patterns was determined per animal, i.e., the difference between pre- and post-injection values expressed as a percentage of the summated pre- and post-injection values:

$$\Delta_{rel} = \frac{X_{post} - X_{pre}}{X_{post} + X_{pre}} \times 100\% \; (X_{pre} \geq 10)$$

The values thus obtained per test group were compared with the corresponding values for the appropriate control group (Mann-Whitney U-test). The difference between this definition of a relative change and the more conventional definition of a relative change,

$$\frac{X_{post} - X_{pre}}{X_{pre}} \times 100\%,$$

should be noted.

RESULTS

Intraseptal Injections 15–16 min After Intraperitoneal Morphine

At this point of time, the morphine-treated cats became more and more active, gradually extending their behavioural activities. Head and limb movements, which were initially performed in a regular, fluent way, had lost their normal character and were replaced by a great number of staccato-like motor elements that rapidly increased in frequency and that were of disorderly character. In general, the cats had adopted a sphinx-like posture moving their heads abruptly from one direction to another and frequently raising, stretching, or shaking their forepaws.

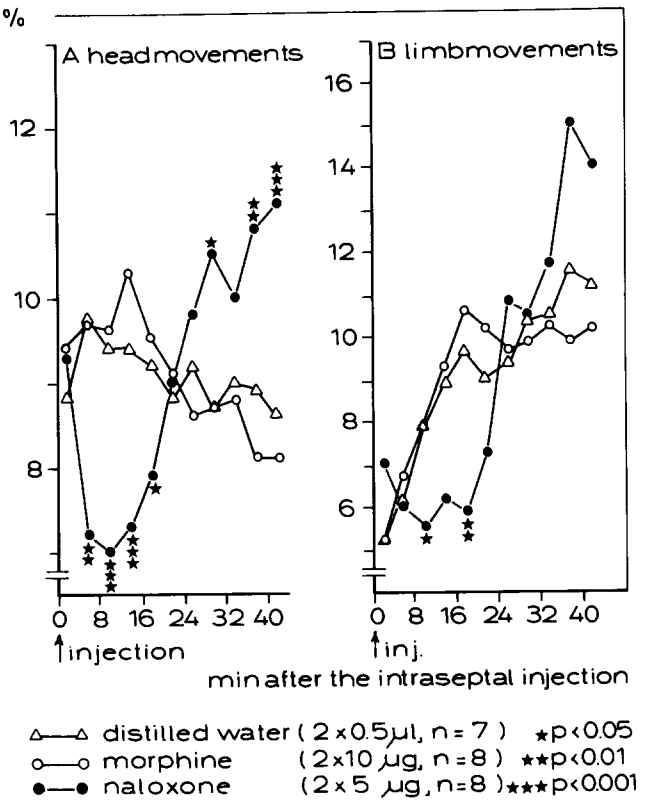

FIG. 1. Behavioural effects caused by intraseptal administration of distilled water, morphine, and naloxone 15–16 min after intraperitoneal administration of morphine. The number of head and limb movements scored per animal per 4 min period is expressed as a percentage of the total number of movements scored for each individual animal during the whole observation period of 44 min immediately following the intraseptal injection. Presented are mean values averaged over each test group. The values obtained for each test group were compared per 4 min-period with those obtained for the control animals (2-tailed Mann-Whitney U-test). For more details see Behavioural Analysis.

Intraseptal injections of naloxone (2×5 µg (15 nmol); n=8) at this point of time caused a decrease in the frequencies of head and limb movements when compared with control injections of distilled water (2×0.5 µl; n=7) as indicated in Fig. 1. This decrease in frequencies of head and limb movements reached a maximum level about 4 min after the intraseptal injections. About 12–16 min later, the frequencies of head and limb movements increased again whereas these frequencies in the control animals had already reached a maximum level at this time (Fig. 1). The naloxone injections did not affect the abnormal nature of the movements, which—although decreased in frequency—remained staccato-like, poorly co-ordinated and of disorderly character.

TABLE 1

BEHAVIOURAL EFFECTS ELICITED BY INTRASEPTAL ADMINISTRATION OF OPIATE AGONISTS 40–41 MIN AFTER INTRAPERITONEAL MORPHINE

Drug Treatment		n	Δ_{abs} (mean ± SEM)
1 Distilled water	2× 0.5 µl	15	6 ± 3‡
2 Morphine	2×10 µg	11	14 ± 7^{ns}
3 Fentanyl	2× 5 µg	10	21 ± 7*
4 Met-enkephalin	2×10 µg	10	21 ± 12^{ns}
5 β-endorphin	2×10 µg	14	31 ± 8†
6 Fentanyl Naloxone	2× 5 µg 2× 5 µg	8	−1 ± 1^{ns,§}
7 β-endorphin Naltrexone	2×10 µg 2× 5 µg	8	11 ± 7^{ns,¶}
8 β-endorphin Naltrexone	2×10 µg 2× 5 µg #	4	14 ± 17^{ns}

Frequencies of locomotor patterns are used as dependent variables. Mean values ± SEM averaged over the whole test group for Δ_{abs} (defined as $X_{post} - X_{pre}$) are given for each drug treatment; for details see Behavioural Analysis.

Drug vs distilled water, 2-tailed Mann-Whitney U-test; *$p<0.05$, †$p<0.02$, ns=not significant, ‡Not tested.

§$p<0.01$ (fentanyl/naloxone vs fentanyl, 1-tailed Mann-Whitney U-test).

¶$p<0.05$ (β-endorphin/naltrexone vs β-endorphin, 1-tailed Mann-Whitney U-test).

#Same drug treatment as for group 7 with the difference that these animals were tested with an intertrial interval of 1 day instead of the usual interval of 14 days.

TABLE 2

BEHAVIOURAL EFFECTS ELICITED BY INTRASEPTAL ADMINISTRATION OF OPIATE ANTAGONISTS 40–41 MIN AFTER INTRAPERITONEAL MORPHINE

Drug Treatment		n	Δ_{rel} (%) (mean ± SEM)
1 Distilled water	2× 0.5 µl	9	13 ± 8‡
2 Naloxone	1× 5 µg	9	−1 ± 9^{ns}
3 Naloxone	2× 5 µg	5	−38 ± 15†§
4 Naltrexone	2× 1 µg	5	−15 ± 13^{ns}
5 Naltrexone	2× 5 µg	8	−29 ± 10†¶
6 Naloxone Morphine	2× 5 µg 2×10 µg	7	−16 ± 8*#

Frequencies of locomotor patterns are used as dependent variables. Mean values ± SEM averaged over the whole test group for Δ_{rel} (defined as $\frac{X_{post} - X_{pre}}{X_{post} + X_{pre}} \times 100\%$) are given for each drug treatment; for details see Behavioural Analysis.

Drug vs distilled water, 2-tailed Mann-Whitney U-test; *$p<0.05$, †$p<0.02$, ns=Not significant, ‡Not tested.

§Not significant (unilateral naloxone vs bilateral naloxone, 1-tailed Mann-Whitney U-test).

¶Not significant (5 µg naltrexone vs 1 µg naltrexone, 1-tailed Mann-Whitney U-test).

#Not significant (naloxone/morphine vs naloxone, 1-tailed Mann-Whitney U-test).

Intraseptal injections of morphine (2×10 µg (31 nmol); n=8) at this time did not affect the behaviour: the frequencies of head and limb movements after the intraseptal injections of morphine were comparable to those following the control injections (Fig. 1).

Intraseptal Injections 40–41 min After Intraperitoneal Morphine

At this stage of the morphine syndrome, the cats displayed a restricted number of senseless, abnormal behaviour patterns, which were regularly repeated in a specific sequence at a stabilized level. These behaviour patterns were individual-specific, e.g., some cats were circling around stationary hind limbs whereas other cats were running from one side of the cage to the other side. The cats neither recognized nor friendly responded to the observer when he was approaching, nor did follow an object moving in front of their eyes.

Opiate agonists. Intraseptal injections of the opioid peptide β-endorphin (2×10 µg (3 nmol); n=14) at this point of time caused an increase in the frequencies of locomotor patterns when compared with control injections of distilled water (2×0.5 µl; n=15) as indicated in Table 1. The overall effect reached a maximum level about 3 min after the intraseptal injections and was still present 16 min later. β-Endorphin did not affect the nature of the stereotyped behaviour patterns: patterns that were already present before the intraseptal injections remained present after the injections, and, moveover, the way in which particular spatio-temporal patterns were built up out of single units remained the same as before the injections. The β-endorphin-induced increase in the frequencies of locomotor patterns was suppressed by intraseptal injections of naltrexone (2×5 µg (15 nmol); n=8) given immediately before the injections of β-endorphin (Table 1). In an additional experiment, in which 4 animals received intraseptal injections of the naltrexone/β-endorphin combination 24 hr after they had received intraseptal injections of β-endorphin alone, a similar result was obtained (Table 1). A remarkable observation, however, was that these 4 animals showed a reduced sensitivity for the second intraperitoneal administration of morphine, i.e., the values for the number of locomotor patterns displayed during the 10 min before the intraseptal injections (X_{pre}) were only 24±14% (mean±SEM) of those observed in the first trial (X_{pre}=70±25).

Intraseptal injections of fentanyl (2×5 µg (9 mol); n=10) also caused a statistically significant increase in the frequencies of locomotor patterns (Table 1). Although the dose of fentanyl was higher than that of β-endorphin, the magnitude of the effect of fentanyl was smaller than that of β-endorphin. Fentanyl did not affect the nature of the stereotyped behavior patterns: patterns that were already present before the intraseptal injections remained present after the injections, and, moreover, the way in which particular spatio-temporal patterns were built up out of single units was the same as before the injections of fentanyl. The fentanyl-induced increase in the frequencies of locomotor patterns appeared immediately after the intraseptal injections and gradually disappeared in the course of the 19 min following the injections. The effect of fentanyl was suppressed by simultaneous intraseptal injections of naloxone (2×5 µg (15 nmol); n=8).

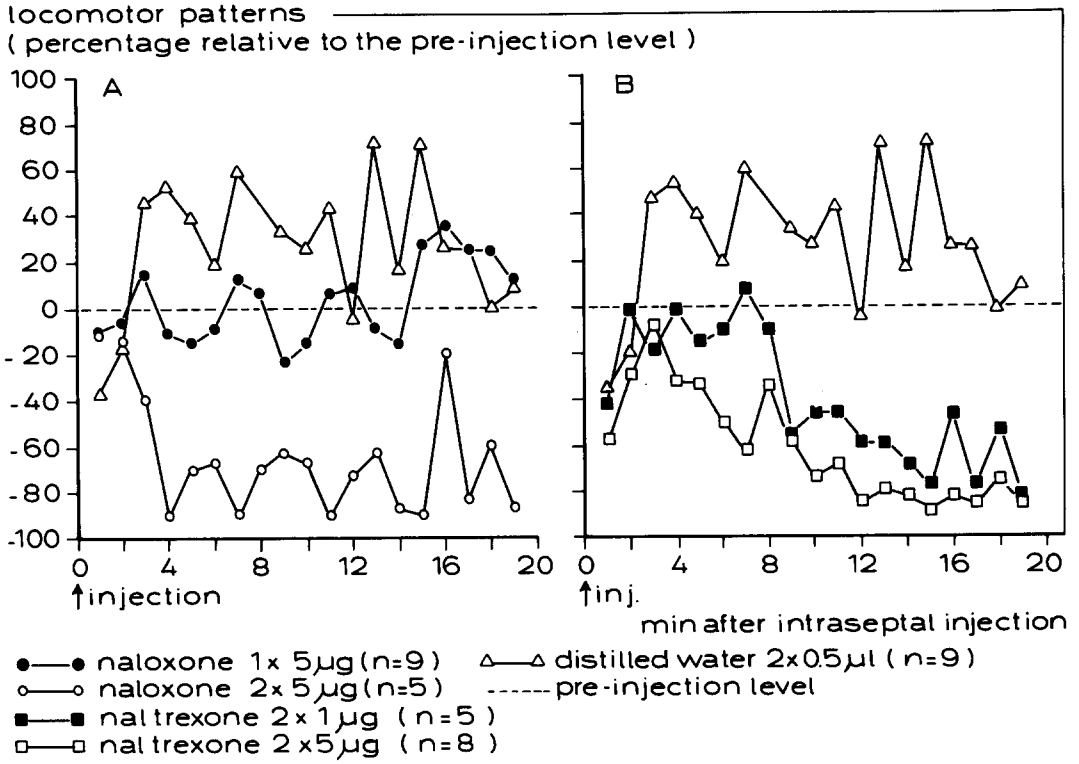

FIG. 2. Behavioural effects caused by intraseptal injection of distilled water, naloxone and naltrexone 40–41 min after intraperitoneal administration of morphine: time-course of the drug-induced effects on locomotor activity. Because of inter-individual differences in locomotor activity, a particular procedure was followed to realize this figure: (1). Use was made of the frequencies of locomotor patterns scored per min during the 10 min immediately preceding the intraseptal injections and during the 19 min immediately following the intraseptal injections. (2). For each individual animal these frequencies were normalized, i.e., the scores per min were divided by the total score over the 10 min preceding and the 19 min following the intraseptal injections. (3). Next, the values obtained in this manner were averaged over the test group. (4). The post-injection values expressed as percentages relative to the pre-injection level are presented.

Neither intraseptal injections of morphine itself (2×10 μg (31 nmol); n=11) nor intraseptal injections of the opioid peptide Met-enkephalin (2×10 μg (17 nmol); n=10) affected the behaviour: the drug-induced changes in the frequencies of locomotor patterns were not statistically significantly different from those following the control injections (Table 1).

Opiate antagonists. Intraseptal injections of naloxone (2×5 μg (15 nmol); n=5) produced a decrease in the frequencies of locomotor patterns in comparison with the control injections of distilled water (2×0.5 μl; n=9) as indicated in Table 2. The behaviour patterns displayed after the naloxone injections were the same as displayed before the injections. Moreover, the behaviour patterns—although less frequently repeated—remained stereotyped, i.e., the way in which the patterns were built up out of single units, was the same as before the injections of naloxone. The overall naloxone-induced decrease in the frequencies of locomotor patterns reached a maximum level (of about 73% inhibition relative to the pre-injection level) about 3 min following the intraseptal injections and was still present 16 min later (Fig. 2A). Unilateral injections of naloxone (1×5 μg (15 nmol); n=9) elicited a less pronounced effect (Table 2; Fig. 2A). Intraseptal injections of morphine (2×10 μg (31 nmol); n=7) did not significantly affect the naloxone-induced decrease in the frequencies of locomotor patterns when administered immediately before naloxone (2×5 μg (15 nmol); Table 2).

Intraseptal injections of naltrexone (2×5 μg (15 nmol); n=8) caused an effect similar to that of naloxone, viz a decrease in the frequencies of locomotor patterns (Table 2). The drug affected the behaviour of the majority of the animals within 3 min; the effect reached a maximum level (of about 84% inhibition relative to the pre-injection level) about 11 min following the injections (Fig. 2B). A lower dose of naltrexone (2×1 μg (3 nmol); n=5) caused a smaller decrease, which also reached its maximum level (of about 67% inhibition relative to the pre-injection level) about 11 min following the injections (Fig. 2B).

Localization of the Injection Sites

The injection sites showed no signs of tissue damage apart from a thin layer of degenerate tissue around the tracks and tips of the cannulas. All injection sites were localized within the anterior part of the septum (i.e., between the stereotaxic co-ordinates A 15.0 and A 18.5; cf. [53]). Table 3 shows in which parts of the septum the injection sites for the effective agents, i.e., for naloxone, naltrexone, fentanyl and β-endorphin, were localized: 66% of the injection sites were

TABLE 3

DISTRIBUTION THROUGHOUT THE SEPTAL NUCLEI OF INJECTION SITES INTO WHICH NALOXONE, NALTREXONE, FENTANYL, OR β-ENDORPHIN WERE INJECTED

Brain Region	Number of Effective Sites (a)	Number of Ineffective Sites (b)	Ratio (a/b)
BD	16	6	2.67
DA	2	1	2.00
DM	2	0	—
H	2	1	2.00
IC	0	1	0.00
LI	1	0	—
MA	69	12	5.75
(MA+1 mm)	(94)	(19)	(4.95)
MP	8	2	4.00

The ratio between the number of effective sites and ineffective sites is given per nucleus.

Abbreviations: BD=nucleus of the diagonal band of Broca pars dorsalis; DA=nucleus septalis dorsalis pars anterior; DM=nucleus septalis dorsalis pars intermedius; H=anterior continuation of the hippocampus; IC=islands of Calleja; LI=nucleus septalis lateralis pars interna; MA=nucleus septalis medialis pars anterior; MP=nucleus septalis medialis pars posterior (Nomenclature according to Andy and Stephan [2]).

localized within the nucleus septalis medialis pars anterior (MA) and a further 26% with 1 mm of it. Furthermore, the ratio effective/ineffective sites was the highest for this part of the septum.

DISCUSSION

The intraseptal injections of opiate agents clearly affected the behavioural response of cats to systemic administration of morphine both during the re-organization and ritualization phases. This observation underlines the important role for septal structures in the development and maintenance of the morphine-induced behaviour of cats, which has been suggested by earlier reports [36, 37, 47]. Still, it has to be concluded that systemically administered morphine does not act directly on septal opiate receptors to produce behavioural effects as will be discussed below. But, first of all, the specificity of the septally evoked effects has to be considered.

The septally evoked effects are most probably due to interactions of the intraseptally injected drugs with opiate receptors since (1) opiate agonists and antagonists elicited mutually opposite effects, i.e., agonists increased and antagonists decreased the frequencies of the behaviour items, and (2) the effects elicited by the opiate agonists could be suppressed by opiate antagonists. Moreover, the effects of the intraseptal injections are most probably mediated via septal structures:

(1) In contrast to manipulation of structures adjacent to the septum such as the caudate nucleus, manipulation of the septum itself only causes changes in the frequencies of behaviour patterns elicited by intraperitoneal administration of morphine, without affecting the nature of the stereotyped behaviour patterns ([11, 13, 36, 37]; Cools and Van Beek, unpublished results). Again, in the present study, only changes in the frequencies of the morphine-induced behaviour patterns have been found after manipulation of the septum.

(2). At least the effect of β-endorphin is not mediated via diffusion into the adjacent ventricular system: the β-endorphin-induced increase in the frequencies of locomotor patterns was observed within 3 min after the intraseptal administration of β-endorphin whereas similar excitatory effects have been reported to occur after a latency of 15–30 min following intraventricular injection of the peptide in a comparable dose [15,38].

(3). The majority of the injection sites were located within or near a restricted part of the septum, viz the nucleus septalis medialis pars anterior. As the ratio effective/ineffective sites was highest for this part of the septum, a foremost conclusion is that receptors within this particular part, rather than septal structures adjacent to it, are essential for the display of the observed phenomena. It is just this anterior part of the septum which has been implicated earlier in the behavioural effects of morphine in rats [29].

A noticeable observation was that the effect of naltrexone reached a maximum level about 11 min after intraseptal injection whereas the effect of naloxone was already maximal about 3 min after intraseptal injection. The lower lipophilicity of naltrexone in comparison to that of naloxone may underly this difference between the effects of both opiate antagonists [22,28].

In order to get more insight into the pharmacological character of septal opiate receptors, we determined the relative effectiveness of intraseptal injections of 4 different opiate agonists (morphine, fentanyl, Met-enkephalin, β-endorphin) during the ritualization phase. Taking into account the applied doses, which were not equivalent on a molar base (62 nmol morphine >34 nmol Met-enkephalin >18 nmol fentanyl >6 nmol β-endorphin), it can be stated that these opiate agonists showed the following ranking order of potencies on septal opiate receptors: β-endorphin >fentanyl >Met-enkephalin >morphine. The 4 opiate agonists show a similar order of potencies at the ε-type, but not at the μ- or δ-types of opiate receptors (see introductory paragraphs; cf. [50, 57, 58]). Therefore, it can be concluded that the septally evoked effects are most probably mediated via ε-type of opiate receptors. Although the presence of δ-receptors and enkephalins within the septum has been demonstrated before [14, 24, 48, 49, 51, 55] and, moreover, the low effectiveness of intraseptal injections of Met-enkephalin may be attributed to its rapid metabolic degradation [21, 25, 33], the observed ranking order of potencies excludes the involvement of δ-receptors in the septally evoked effects since fentanyl, being more potent than β-endorphin at δ-receptors (see introductory paragraphs), turned out to be less potent than β-endorphin the present study.

The tentative conclusion that septal opiate receptors of the ε-type are involved in the septally evoked effects is corroborated by the findings of other authors: intraseptal injections of β-endorphin are far more effective than intraseptal injections of morphine in decreasing the hippocampal turnover rate of acetylcholine [40,41]. Moreover, high levels of β-endorphin (which may be indicative for ε-receptors) are present within the septum [4, 30, 48] whereas the levels of enkephalins (which may be indicative for δ-receptors) are only moderate [24, 48, 49, 51, 55] and the density of μ-receptors even small [3,43] within the septum.

The possible involvement of the ε-type of opiate receptors, which is rather insensitive for morphine, may explain the failure of intraseptal injections of morphine to affect (1)

the frequencies of head and limb movements during the reorganization phase, (2) the frequencies of locomotor patterns during the ritualization phase, (3) the effect of naloxone during the ritualization phase, and (4) the behaviour of cats not pretreated with a systemic injection of morphine (present study; Megens, unpublished results). Of course, it might be possible that higher doses of intraseptally applied morphine would be effective. Higher doses were not applied, however, as morphine was already injected in the highest dose compared to those used for the other agents. Furthermore, the use of high doses would have resulted in a greater spread of the injected drugs [5] and, consequently, in less certainty about the locus specificity of the evoked effects.

The tentative conclusion that the septally evoked effects are mediated via ε-type of opiate receptors implicates that systemically administered morphine does not act directly on these receptors to produce behavioural effects. On the other hand, the present results demonstrate that intraseptal injections of opiate agonists and antagonists respectively potentiate and suppress the behavioural effects of systemically applied morphine. Therefore, when systemic morphine does not act directly on septal opiate receptors, the present results imply that it influences indirectly these receptors. The septum receives its endorphinergic afferents from the hypothalamus [4, 48, 56]. Hence, it is possible that systemic administration of morphine results in an increased release of β-endorphin within the septum via an action on the endorphinergic, hypothalamo-septal neurons, thereby indirectly stimulating septal, ε-type of opiate receptors. Indeed it has previously been demonstrated that chronic administration of morphine affects the level of β-endorphin within the septum [45]. A similar situation appears to exist in the pituitary: systemic administration of morphine results in an increased release of adrenocorticotrophic hormone (ACTH) and, as β-endorphin is released concomitantly with ACTH [1, 20, 54], also of β-endorphin from the pituitary via an action on the hypothalamus [16, 19, 39, 52].

As a final remark, which has already been mentioned in the Results section, intraseptal injection of β-endorphin apparently reduced the sensitivity of the cats to a second injection of morphine 24 hours later, suggesting that septal opiate receptors of the ε-type are not devoid of a possible role in the development of tolerance (cf. [29,45]. Summarizing, the most important conclusions of the present study are (1) systemically administered morphine does not affect behaviour via a direct action on septal opiate receptors, and (2) the receptors mediating the septally evoked effects are most probably ε-type opiate receptors.

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