MORPHINE WITHDRAWAL SYNDROME: DIFFERENTIAL PARTICIPATION OF STRUCTURES LOCATED WITHIN THE AMYGDALOID COMPLEX AND STRIATUM OF THE RAT

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SUMMARY

The participation of amygdaloid and striatal structures in the various signs of morphine withdrawal syndrome (MWS) has been investigated using two complementary approaches: intracerebral application of naloxone in various nuclei of dependent rats and effects of various lesions and transections on systemically induced MWS. In morphine dependent rats local application of naloxone (10 μg in 0.1 μl solution) in the vicinity of either the central nucleus of the amygdala or the lateral anterior nucleus but not in adjacent amygdaloid or striatal nuclei elicited the jump sign. Diarrhea was more frequently elicited by application of naloxone in the striatum or centromedial amygdala than the basolateral parts of the complex. In contrast, there was no specific anatomical correlates to the remaining signs including wet shakes, paw tremor, teeth chattering and chewing.

Bilateral electrolytic lesion of the central amygdala or combined transection of the stria terminalis and so-called ventral amygdalofugal pathway eliminated the jump without affecting the remaining signs. In contrast a large bilateral destruction of the entire striatum did not significantly affect the various signs. Bilateral transection of the stria terminalis reduced only the occurrence of the wet shakes. Also, a transection of the medial forebrain bundle considerably reduced the 3 main signs, i.e. jump, wet shakes and diarrhea. These results suggest that, in analogy to the acute actions of morphine, MWS should not be considered as a unitary phenomenon but as an ensemble of signs which probably reflect the intense activation induced by naloxone in a number of brain sites consequent to the abrupt interruption of opioid actions in a dependent animal. The significance of these results is discussed with reference to previous anatomical, biochemical and behavioural studies performed on the amygdala.
INTRODUCTION

Morphine dependent animals manifest a characteristic autonomic and behavioural syndrome when morphine intake is abruptly terminated or opioid antagonists systemically injected\(^9,14,44,63\). The complex character of this morphine withdrawal syndrome (MWS) which includes diarrhea, paw tremors, teeth chattering, wet shakes, chewing, writhing, so-called 'jumps' or leaping attempts to escape from the jar and various other signs (ibid) suggests a central nervous system involvement. It also suggests that different brain structures which contain opioid endogenous ligands and their receptors and also have autonomic and behavioural regulating roles may be affected by the abrupt interruption of morphine action. However, little information is present in the literature concerning the neuroanatomical correlates of the various signs which constitute MWS (see Discussion).

In the present report, we have examined the participation of the amygdaloid complex and the striatum in MWS and in particular tested the hypothesis that adjacent nuclei may differentially intervene in the various signs of MWS. The study was centred on the amygdaloid complex since (a) it participates in the regulation of a large number of autonomic and behavioural functions and electrical stimulation of the amygdala elicits various motor and vegetative symptoms, some of which are reminiscent of withdrawal signs (see refs. 21, 31, 66); (b) localized amygdaloid nuclei are among the richest brain structures in both opiate receptors and their endogeneous ligands (see refs. 2, 25, 34, 55); (c) this abundance of amygdaloid opioid systems is poorly understood; it may be related to the cataleptic\(^{15}\) and epileptic\(^{50,56}\) features of opiate administration. In the present report, which is part of a multidisciplinary approach centred on the amygdala\(^3,6,8,39,50,\) using two complementary approaches, namely the local application of naloxone in various nuclei of morphine dependent rats and the effects of various lesions on systematically induced MWS, it is shown that the central amygdala is involved in the jump sign of MWS. Part of these results have been published previously\(^{11,35}\).

MATERIALS AND METHODS

Two hundred and thirty-one male Wistar rats (250–300 g) were used in the experiments. They were given food and water ad lib and were housed under diurnal lighting conditions with lights on from 08.00 to 20.00 h.

In the first experimental series (\(n = 92\) rats), the animals were anaesthetized with Equithesin (4 ml/kg, Jensen–Salsbury), placed in a stereotaxic frame and unilaterally implanted with a stainless steel guide cannula (0.3 mm i.d., 0.38 mm o.d.). The tip of the cannula was placed 1 mm above the target structure relying on the atlas of Albe-Fessard et al.\(^1\). After a recovery period of one week, morphine dependence was elicited in 85 rats by subcutaneous (s.c.) injections of a sustained release preparation of morphine according to the technique of Collier et al.\(^{14}\) with minor modifications: morphine was suspended in 4.25 ml of liquid paraffin and 0.75 ml of mannide monooleate (Arlacel A), an emulsifying agent. This oily mixture was emulsified with 5
ml of 0.9% w/v NaCl in water. The first day, a 50 mg/10 ml/kg solution was injected s.c.; a solution of 120 mg/10 ml/kg was administered s.c. 24 h later. MWS was precipitated 24 h later by an intracerebral injection of naloxone (10 µg diluted in a 0.1 µl NaCl solution of 0.9% w/v). The injection was made in the unanaesthetized animal via a delivery cannula (0.18 mm i.d., 0.28 mm o.d.) in approximately 1.5 min. The delivery cannula was 1 mm longer than the previously implanted guide cannula to just reach the target structure. The delivery cannula was left at the site of injection for 5 min approximately.

Following naloxone administration, each animal was placed in a plexiglass cylinder (35 cm diameter, 130 cm height) and observations were made during a period of 30 min after the injection of naloxone. The incidence of the following withdrawal signs was counted (see refs. 9, 14, 44, 63): 'jump', wet shake, paw tremor, chewing and teeth chattering. The criteria we used for these symptoms were one or more for the first sign and three or more for the wet dog shakes. Diarrhea and other signs such as penile erection and licking as well as ptosis were continuously noted during the 30 min epoch.

The control experiments for this experimental series included intracerebral injection of naloxone in similar conditions in animals which had received the emulsion s.c. without morphine (n = 7 rats), intracerebral injection of the vehicle solution (NaCl) in morphine dependent rats (n = 4) and systemic administration of naloxone in morphine dependent rats (see below).

In the second experimental series (n = 139 rats), the animals were anaesthetized (see above), placed in a stereotaxic frame and one of the following brain lesions bilaterally performed (see Figs. 1 and 4).

(A) Lesion of the central amygdala (n = 34 rats): using a radionic temperature controlled electrode, the lesion was centred at the coordinates A5.8, L4.25, H2 (ref. 1).

(B) Lesion of the striatum (n = 8 rats): using the same device, the lesion was centred at coordinates A7, L4, H4.5 (ref. 1).

(C) Lesion of the dorsal striatum (n = 16 rats): using the same device, the lesion was centred at coordinates A7, L4, H6 (ref. 1).

(D) Transection of the stria terminalis (n = 26 rats): a portion of a razor blade (2 mm width) was glued to a conventional electrode holder and stereotaxically introduced to the coordinates A.5.8, L3, H5 (see Fig. 1 in ref. 5).

(E) Combined transection of the stria terminalis and the ventral amygdalofugal (VAF) pathways (n = 7 rats): using a remote controlled leucotome this procedure permits complete transection of both major afferent and efferent pathways of the amygdala (see ref. 5 for further details).

(F) Transection of the medial forebrain bundle (MFB) (n = 16 rats): the remote controlled leucotome was vertically introduced under stereotaxic guidance to the coordinates A5.6, L1.25, H2 (ref. 1). The blade, locked in the probe during the descent, was oriented in the medial direction at 90° and brought back to its locked position before withdrawing the leucotome. This procedure transects the MFB with relatively limited damage to the structures traversed by the tract (see Fig. 1); there was no loss in weight and no apparent motor disturbance a week after the transection. In contrast, in preliminary experiments, most of the animals (n = 14) died a few days following a bilateral electrolytic lesion of the MFB.
Fig. 1. Bilateral transection of the medial forebrain bundle (MFB) with a remote controlled leucotome (see ref. 13). Photomicrographs showing three consecutive 100 µm thick frontal sections, Nissl stain. Note the gliosis in the hypothalamus and MFB induced by the blade and along the descent of the leucotome. Little injury was apparent in rostral or caudal sections in the hypothalamic area.
After a recovery period of one week, morphine dependence was elicited as described above and withdrawal precipitated by i.p. administration of naloxone (1 mg/kg or 5 mg/kg; n = 30 rats, see below). As previously described, the withdrawal signs were counted during a period of 30 min. Control animals received s.c. morphine similarly and MWS was precipitated with i.p. naloxone (1 mg/kg, n = 16; 5 mg/kg, n = 16). It is worth stressing that in both experimental series, MWS was usually precipitated between 14.00 and 18.00 h.

At the end of withdrawal syndrome examinations, in both experimental series, the animals were deeply anaesthetized and 0.2 µl of Pontamine sky blue injected via the cannula in the chronically implanted group. The animals were then intracardially perfused with saline followed by a 10% formalin solution. Each brain was then cut on a freezing microtome and the frozen sections stained with cresyl violet and mounted on glass slides. Each slide was then projected on to a screen and photographs taken from those containing cannulae or lesion traces. These procedures allowed identification of the position of each cannula track, the extent of diffusion of 0.2 µl Pontamine sky blue (see Fig. 2 and ref. 35), and the extent of the lesions or transections in the second experimental series.

Fig. 2. Histological control of the localisation of the delivery cannula and extent of diffusion of locally applied naloxone. At the end of the behavioural examination, 0.2 µl Pontamine sky blue was locally injected via the delivery cannulae and conventional histological procedures performed. The photomicrographs A and B show in two different animals the diffusion of Pontamine blue. Abbreviations for this and following figures: ac, central nucleus; aco, cortical nucleus; alp, lateral posterior nucleus; am, medial nucleus; cp, caudate putamen; cpf, pyriform cortex; TO, optic tract.
RESULTS

Intracerebral injection of naloxone in morphine dependent rats

In morphine dependent rats, unilateral intracerebral injection of naloxone elicited withdrawal signs similar to those observed after systemic application of the antagonist in the control group (see refs. 9, 14, 63 and below). Relying on the histological controls, the localization of each delivery cannula and the extent of diffusion of Pontamine sky blue (see Fig. 2) were precisely determined and the efficiency of each site of injection to elicit a particular withdrawal sign was plotted on

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Fig. 3. Anatomical localization of sites from which naloxone elicited the jump 'sign' in morphine dependent rats. MWS was elicited by unilateral intracerebral injections of naloxone through a stainless steel delivery cannula (see text). Note that the jump sign (●, efficient sites) was almost exclusively observed when the cannulae were located either in the vicinity of the central nucleus (ac) or in the dorsolateral part of the lateral-anterior nucleus (ala). In contrast this particular withdrawal sign was seldom observed (▲, non-efficient sites) following applications of naloxone in other amygdaloid or striatal structures. The figures are drawn from the atlas of König and Klippel[3]; the anterior level of each section is indicated. Abbreviations (see also Fig. 2): ala, lateral anterior nucleus; abm, basomedial nucleus; abl, basolateral nucleus; CAE, capsula externa; cl, claustrum; GP, globus pallidus; MI, massa intercalata; ol, nucleus of the lateral olfactory tract.
naloxone elicits diarrhea

naloxone does not elicit diarrhea

Fig. 4. Anatomical localization of sites from which naloxone elicited diarrhea in morphine dependent rats. Note that diarrhea was elicited (○, efficient sites) primarily when naloxone was applied in the central corticomedia1 amygdala or the striatum but seldom in the basolateral amygdala (▲, non-efficient sites).

The jump sign was almost exclusively induced when naloxone was applied either in the central amygdala (ac) or in the dorsolateral tip of the lateral anterior nucleus (ala); injection of the opiate antagonist in the remaining amygdaloid or striatal nuclei did not elicit this particular sign. This is also reflected from Table I in which 5 experimental groups were classified according to the site of injection of naloxone; thus, the jump sign was elicited in 13 out of 16 cases and in 5 out of 5 cases in which the cannulae were located in the vicinity of ac and ala respectively. As in the control animals which received 1 mg/kg naloxone i.p. (see Table I), the rats made one or more leaping attempts to escape from the chamber;
the animal would jump often at a height of 80–100 cm from the ground (see refs. 9 and 36). In contrast, this particular sign was seldom induced by local applications of naloxone in other amygdaloid nuclei (3/38) or in the adjacent striatum, i.e. caudate putamen and globus pallidus (2/22).

Furthermore, as shown in Fig. 4 and Table I, the diarrhea was more frequently elicited following injections of naloxone in the central (12/16) or corticomedial (12/21) than in the basolateral (3/17) parts of the complex (note, the basolateral part includes the lateral posterior and the basolateral nuclei). This particular sign was also readily induced by local injections of the opiate antagonist in the dorsal tip of ala (4/5) or in the striatum (18/22). Furthermore, although more difficult to quantify than motor signs, the diarrhea was particularly abundant after injections of naloxone in the striatum. In contrast, independently of the site of intra-amygdaloid or striatal injection, naloxone elicited the remaining withdrawal signs including wet shakes, paw tremor, teeth chattering, chewing, penile erection and licking.

The following control experiments were performed: in 7 naive rats, naloxone applied locally — including the central nucleus — occasionally elicited chewing or teeth chattering but none of the remaining signs were observed; similar results were obtained in 4 dependent rats, following local application in the central amygdala of the vehicle solution.

Effects of bilateral lesions on morphine withdrawal syndrome

Bilateral lesion of the central amygdala completely eliminated the jump sign induced in morphine dependent rats by 1 mg/kg naloxone i.p. (Table II): in contrast, the remaining signs were not affected by the lesion. Upon histological examination (see Fig. 5A), this lesion always included a complete destruction of the central nucleus; to various extents, in different rats, the adjacent dorsal part of the basomedial nucleus or ventralmost parts of the caudate-putamen were also affected by the electrolytic

TABLE I
Withdrawal signs induced by intracerebral injection of naloxone in morphine dependent rats

Differential effects of unilateral intracerebral injection of naloxone on morphine withdrawal signs according to the site of injection as revealed by the histological examination of the localization of the delivery cannula. The animals were divided in 5 groups (see Figs. 3 and 4). In this and Table II, the number of animals showing each sign according to the criteria (see Methods) is indicated versus the total number of animals in each group.

<table>
<thead>
<tr>
<th>Withdrawal sign</th>
<th>Central amygdala</th>
<th>Lateral anterior nucleus</th>
<th>Corticomedial amygdala</th>
<th>Basolateral amygdala</th>
<th>Striatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jump</td>
<td>13/16</td>
<td>5/5</td>
<td>1/21</td>
<td>2/17</td>
<td>2/22</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>12/16</td>
<td>4/5</td>
<td>12/21</td>
<td>3/17</td>
<td>18/22</td>
</tr>
<tr>
<td>Wet shakes</td>
<td>10/16</td>
<td>4/5</td>
<td>12/21</td>
<td>12/17</td>
<td>15/22</td>
</tr>
<tr>
<td>Paw tremor</td>
<td>14/16</td>
<td>5/5</td>
<td>15/21</td>
<td>15/17</td>
<td>20/22</td>
</tr>
<tr>
<td>Teeth chattering</td>
<td>15/16</td>
<td>5/5</td>
<td>21/21</td>
<td>17/17</td>
<td>22/22</td>
</tr>
<tr>
<td>Chewing</td>
<td>16/16</td>
<td>5/5</td>
<td>21/21</td>
<td>17/17</td>
<td>22/22</td>
</tr>
</tbody>
</table>
Fig. 5. Extent of the damage induced by the electrolytic lesions. A: lesion of the central amygdala; B: lesion covering the entire striatum; C: lesion of the dorsal striatum and adjacent cortex. See text for further explanations.

destruction. In contrast, a large bilateral lesion covering the entire striatum (see Fig. 5B) reduced the jump sign to a much lesser extent than the central amygdaloid injury (see Table II). Since this large lesion included partial damage to amygdalofugal and amygdalopetal systems (see below), in an additional experimental series the lesion was centred on the dorsal part of the caudate-putamen (see Fig. 5C). The latter procedure which destroyed, in addition to the dorsal caudate-putamen, the adjacent corpus callosum and neocortical areas, did not affect the stria terminalis or VAF. As shown in

TABLE II

Effects of various bilateral lesions on morphine withdrawal signs

Differential effects of various bilateral lesions or transections on morphine withdrawal syndrome. MWS was induced a week after bilateral brain injury (see Methods) and withdrawal precipitated in the dependent rats by administration of naloxone (1 mg/kg, or 5 mg/kg i.p. as noted in the table). The control group received similar doses of morphine and naloxone. Note the elimination of the jump sign by lesion of the central amygdala. The asterisk indicates significant difference from respective control ($P < 0.001$, $z^2$ test).

<table>
<thead>
<tr>
<th>Withdrawal signs i.p. Naloxone dose (mg/kg)</th>
<th>Control</th>
<th>Bilateral lesions or transections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Central amygdala</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jump</td>
<td>14/16</td>
<td>16/16</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>14/16</td>
<td>15/16</td>
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<tr>
<td>Wet shakes</td>
<td>12/16</td>
<td>0/16</td>
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<tr>
<td>Paw tremor</td>
<td>15/16</td>
<td>12/16</td>
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<tr>
<td>Teeth chattering</td>
<td>14/16</td>
<td>12/16</td>
</tr>
<tr>
<td>Chewing</td>
<td>16/16</td>
<td>13/16</td>
</tr>
</tbody>
</table>
Table II, this lesion did not affect the incidence of the jump sign but significantly reduced the wet shakes and diarrhea. Thus, in agreement with the intracerebral naloxone experiments, these results suggest that the central amygdala is primarily involved in the jump sign.

Since the central amygdala is traversed by most afferent and efferent systems to the amygdaloid complex (see ref. 37) and in order to investigate the fibre tracts which may be involved in MWS, in an additional experimental series, we have examined the effects of various transections on MWS. As shown in Table II, whereas bilateral transection of the stria terminalis had no effects on the incidence of the jump sign, a transection of both the latter fibre tract and the VAF almost completely eliminated this particular sign. The jump was also absent in animals in which the MFB had been transected. Furthermore, Table II also shows that both the MFB and VAF may also be necessary for the manifestation of the diarrhea. In contrast, three of the remaining signs, i.e. chewing, teeth chattering and paw tremor were not affected by the transections (or lesions, see Table II) with the exception of the latter sign which was slightly decreased by an injury of the MFB.

The effects of the various lesions on the occurrence of wet shakes were more complex. As shown in Table II, the most obvious difference between the control group and the experimental ones was the decrease of the number of animals reaching the criterion for this sign (see Methods) following an injury of the MFB, stria terminalis or dorsal caudate-putamen and adjacent neocortical structures. Furthermore, within the three remaining experimental groups a difference was noted in the frequency of occurrence of this sign. Thus, during the 30 min withdrawal epoch, the animals with a central amygdalectomy and stria terminalis VAF destruction displayed wet shakes at a mean frequency of 9.6 and 12.9 respectively; (the animals which displayed less than 3 wet shakes (see Methods) are not included). These values were of 5.7 and 4.9 for the control and striatal groups respectively.

Effects of higher doses of naloxone (5 mg/kg i.p.) on the MWS in bilateral central amygdalectomized rats

In agreement with Blasig et al., increasing the dose of naloxone given to precipitate withdrawal induced a considerable increase in the occurrence of the jump which was present in every animal (see Table II); the mean number of jumps induced during the 30 min epoch was 25.5 (in comparison with the value of 8.0 for the 1 mg i.p. group). In further agreement with the aforementioned authors, the wet shakes were completely suppressed by the higher naloxone dose (see Table II).

In morphine dependent rats with a central bilateral amygdalectomy, a dose of 5 mg/kg naloxone only partly restored the jump sign (6/14, mean of 19.8 jumps). It is of interest to stress that the wet shakes were present in 9 out of 14 animals (mean frequency of 4.9).

DISCUSSION

The results of the present study suggest that adjacent brain structures and their
associated afferent or efferent pathways may differentially participate in the various signs of morphine withdrawal syndrome. Therefore, MWS should not be considered as a unitary phenomenon but as an ensemble of signs which probably reflect the intense activation induced by naloxone in a number of specific brain sites consequent to the abrupt interruption of opioid actions in a dependent animal (see below and refs. 38, 42 and 63). In the discussion to follow, we shall successively examine the intracerebral injections and lesion experiments before evaluating the significance of the present results in reference to previous anatomical, biochemical or behavioural studies performed on the amygdaloid complex.

Intracerebral application of naloxone in morphine dependent rats

The multiplicity of brain sites mediating the wide spectrum of actions induced by acute opiate administration has been demonstrated by a large number of previous investigations43,52,58. In regard to the site of action of chronic administration of morphine and withdrawal signs, previous studies performed in the monkey19, rabbit27 and rat58,61 have demonstrated that dependence on morphine and withdrawal signs can be induced by intracerebroventricular (i.c.v.) application of the opiate and its antagonists respectively. Recently, Wei et al.62 have reported that, in morphine dependent animals, wet shakes and jumps are most frequently elicited by local application of naloxone in the 'medial thalamic and medial areas of the diencephalic-mesencephalic junctures'. However, in these studies, it has not been possible to localize with some accuracy the individual brain nuclei which may be differentially implicated in the various signs of MWS. This may well be due to the difficulty in restricting the actions of the agent following i.c.v. injections to individual brain nuclei and to the particularly high amounts of naloxone injected in the study of Wei et al. (40–200 μg crystalline naloxone, see ref. 62).

The present results indicate that although several amygdaloid and striatal structures are involved in withdrawal the central nucleus seems to be more implicated in the jump. The small volumes and amounts of naloxone injected, the large number of adjacent non-efficient sites as well as the lesion experiments (see below) suggest that these results are not due to a special vascularization of the central amygdala with a consequent distant or systemic action of the locally applied naloxone.

It is also difficult to reconcile with the latter possibility the selective efficiency of naloxone to elicit diarrhea from only some brain sites. With regard to the remaining signs, in particular the wet shakes which is a recessive sign (see ref. 9), further studies using smaller doses (and volumes) of naloxone must be performed to better evaluate the lack of anatomical correlates suggested by our intracerebral experiment.

Effects of bilateral lesions and transections on morphine withdrawal signs

Previously, various lesion experiments have been performed to examine the central sites related to morphine withdrawal15,65. More recently, Kerr and Pozuelo (ref. 32, also see ref. 24) have reported that the various signs of MWS are reduced by electrolytic lesions of the hypothalamus. These effects may well be due to the destruction of the medial forebrain bundle, since in the present report a transection of
this pathway, which was accompanied by little damage to the structures traversed by
the bundle, considerably reduced the main 'specific' withdrawal signs we have
examined including the jump; our lesion experiments suggest that the central
amygdala and amygdalofugal or petal systems present in the MFB and VAF may be
important for the induction of this particular sign which is usually considered to
reflect a particularly severe withdrawal syndrome. With regard to the diarrhea,
both the lesion and intracerebral experiments suggest that brain structures may well
participate in this particular sign which perhaps is not entirely peripheral, i.e. a
consequence of the presence of opiate receptors in the intestinal tract. This is also
consistent with the view that the constipating effects of morphine are largely due to a
central action with a visceral efferent component.

Behavioural, anatomical and neurochemical correlates of the participation of the
amygdala in morphine withdrawal syndrome

As emphasized in the Introduction, electrical stimulation of the amygdala
induces a large number of autonomic, motor and behavioural reactions including, for
instance, changes in gastro-intestinal secretion and motility, chewing, salivation,
penile erection, flight and defense behaviour. There is some accordance
between the 'functional representation', (see ref. 31) suggested by these studies and the
effects of local application of naloxone in dependent rats. Thus, an increase in gastro-
intestinal motility is readily obtained by stimulation of the anterodorsal and centro-
basolateral parts of the amygdaloid complex (refs. 31, 54) or of the striatum (see
review in ref. 17). With regard to the jump, it is perhaps relevant to note that flight and
defense reactions are most readily induced by electrical stimulation of the central and
dorsal amygdala. However, the comparison between the jump and the aforemen-
tioned reactions must await behavioural studies concerning the significance of the jump
(note, in our hands, during withdrawal, when the animals are placed in an open field,
they display a series of intense particularly rapid 'exploratory' behaviour accompanied
by rearing phases; they start jumping when replaced in the chamber; also see ref. 36).

Since the central nucleus is traversed by most afferent and efferent pathways to
the amygdala, particular caution must be exerted in the interpretation of electrical
stimulation experiments. In that regard, it is of interest to note that local application in
the central amygdala of kainic acid, the potent neurotoxic analogue of glutamate,
rapidly elicits a series of typical focal amygdaloid seizures as well as various signs
including paw tremors, wet shakes and in several occasions jumps and diarrhea. Therefore, intense activation of central amygdala cell bodies can elicit withdrawal
signs.

Several anatomical observations are also of direct relevance to the present
results. Thus, confirming the pioneering study of Dell and Olson, several anatomical
studies have shown that in contrast to adjacent amygdaloid nuclei the central nucleus
receives an abundant afferent innervation from brain structures which directly
participate in the regulation of somatomotor and autonomic functions. At the efferent side, Hopkins and Holstege have recently demonstrated that these structures are also reciprocally innervated by a central amygdalofugal system.
Through this efferent system, which probably follows the VAF route and not the stria terminalis\textsuperscript{28}, the central nucleus can directly control various structures which have direct or indirect connections with somatomotor and visceral nuclei of the cranial nerves\textsuperscript{29}.

With regard to the neural putative transmitters involved in these connections (also see refs. 46 and 53), it is of interest to note the high contents of dopamine (refs. 7, 60, also see refs. 12 and 49) and met-enkephalin of the central nucleus\textsuperscript{25,55}. This contrasts with the opiate receptors which are more homogeneously distributed within the amygdala with, however, a particularly high concentration in an area corresponding to the tip of the ala from which the jump was elicited in the present study by naloxone\textsuperscript{2}. Although the corresponding afferent met-enkephalinergic source is not known (see refs. 25, 59) it is highly significant that several of the aforementioned brain stem and pontine structures are particularly rich in met-enkephalin containing neuronal elements\textsuperscript{55}. In a more general perspective, it is worth noting that Costall and Naylor\textsuperscript{13} have shown that a lesion of the central nucleus and not that of the adjacent striatum abolishes the catalepsy induced by morphine administration suggesting that dopaminergic and opioid systems, in the central amygdala and striatum, may well differentially intervene in the somatomotor effects of acute or chronic administration of morphine (also see, however, ref. 57).

To conclude, the present results suggest that amygdaloid opioid systems are likely to play an important role in mediating the effects of morphine on the somatomotor and vegetative responses associated with emotional behaviour. In a more general perspective, the parallelism between the effects of electrical stimulation, local application of kainic acid or naloxone in dependent rats suggest that during abrupt morphine withdrawal there is an intense increase in neuronal excitability, in opposition to the decrease induced by acute morphine injections (see ref. 47). It is conceivable that in limbic structures such as the amygdala, which are particularly sensitive to epileptogenic inducing procedures or agents\textsuperscript{22,24}, this intense activation may lead to secondary pathological changes such as those seen during alcohol withdrawal\textsuperscript{26,30}. In this respect, it is highly significant to note that both anticonvulsant administration and amygdaloid kindling procedure considerably decrease the severity of MWS\textsuperscript{20,41}.

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