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Reversal of Morphine-Induced Catalepsy by Naloxone Microinjections into Brain Regions with High Opiate Receptor Binding: A Preliminary Report

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WILCOX, R. E., M. BOZARTH AND R. A. LEVITT. *Reversal of morphine-induced catalepsy by naloxone microinjections into brain regions with high opiate receptor binding: A preliminary report.* PHARMACOL BIOCHEM BEHAV **18**(1) 51–54, 1983.—The relationship between opiate binding density and morphine-induced catalepsy was estimated via dose-response analysis of the brain sites in which naloxone microinjections reversed the catalepsy induced by intraperitoneal morphine. One-hundred forty-one experimentally naive male Long-Evans rats were implanted with chemical microinjection guide cannulae aimed for various high-to-moderate binding density areas within caudate nucleus, central gray matter, thalamus, hypothalamus, amygdala, and frontal cortex as well as low density sites in pyriform cortex and various fiber tracts. Overall, 48 out of 91 animals microinjected with naloxone in brain sites having high-to-moderate density of opiate binding showed reversal of the cataleptic response. Dose-response effects were found in all 6 high-to-moderate density sites: ranging from 85% reversals at 100 mcg naloxone over all sites to 34% reversals at 0.01 mcg naloxone. There were no reversals out of 38 naloxone microinjection in brain sites having a low density of opiate binding and no reversals out of 18 saline microinjections in either high-to-moderate or low opiate binding density loci. These results suggest a role for limbic and basal ganglia portions of the opiate system in a motor aspect of narcotic action. We speculate that these loci may also play a role in the motor expression of the response to the analgesic and euphoric actions of morphine to supplement actions mediated through periventricular structures.

Catalepsy Morphine Opiate receptor Naloxone Motor system Frontal cortex Caudate nucleus Thalamus Hypothalamus Amygdala Periaqueductal gray matter

THE reports demonstrating stereospecific, high affinity binding of opiate drugs to subcellular fractions from tissue from several regions of mammalian brain [6,15] provided an important tool with which to probe the neuroanatomical substrate of the actions of morphine [9]. In several species the highest concentrations of such ligand-binding defined opiate receptors are found in limbic system and basal ganglia loci. An especially rich distribution is found in a group of periventricular core structures including periaqueductal gray, midbrain reticular formation, and medial hypothalamus and thalamus, comprising a spinoreticularpaleospinothalamic system implicated in the processing of nociceptive information [6,10]. An important focus of research on opioid drugs is the separability of the various actions of morphine with a goal of developing a narcotic analgesic with reduced potential for self-administration, tolerance development and physical dependence [11]. One useful approach for determining the neuroanatomical substrate for opioid actions has been the correlation between microinjections of narcotic agonists or antagonists into specific brain loci with behavior [5, 8, 17, 22].

A potential limitation of early work on the neural substrate for narcotic actions is that narcotic analgesics have a profound influence on general sensorimotor processing in addition to their analgesic-euphoric effects [1,20]. Furthermore, as commonly measured, analgesia and physical dependence induced by morphine involve changes in the motor output of the organism [5, 23, 24]. A clarification of the brain areas participating in opiate-induced changes in general sensorimotor function may help to separate these responses from analgesia-euphoria and dependence.

Previous studies have implicated structures such as the caudate nucleus in the rigidity [4] catalepsy [24] but not the analgesia [20] associated with morphine administration. The

TABLE 1
NALOXONE REVERSAL OF MORPHINE CATALEPSY

	Naloxone dose (mcg)							
	Saline		0.01		0.1		100	
	Mean	Reversal	Mean	Reversal	Mean	Reversal	Mean	Reversal
High density sites*								
Caudate (27)†	30	0% (3)	23.8	25% (12)	18.6	43% (7)	3.0	100% (5)
Central gray (16)	30	0% (2)	17.7	57% (7)	6	100% (1)	5.8	100% (6)
Thalamus (14)	30	0% (1)	22.3	33% (3)	16.0	60% (5)	12.0	60% (5)
Hypothalamus (9)	30	0% (1)	15	50% (4)	5	100% (1)	4.5	100% (3)
Amygdala (14)	30	0% (2)	30	0% (3)	20.5	33% (6)	0	100% (3)
Frontal cortex (23)	30	0% (3)	—	—	30	0% (9)	6.7	82% (11)
Low density sites Pyriform cortex (6) Fiber systems (32)	30	0% (6)	30	0% (14)	30	0% (14)	30	0% (4)

*Binding classifications were made as follows. Loci for which binding data were available for rat brain were designated as high-to-moderate binding if they had 5.0 or more autoradiographic grains of diprenorphine in 100 square microns of tissue or low binding with fewer than 5.0 [14]. Loci for which binding data were available for rhesus monkey brain were designated as high-to-moderate binding if they had 5.0 or more fmol dihydromorphine binding/mg protein or low binding with fewer than 5.0 [10]. Loci for which binding data were available for human brain were designated as high-to-moderate binding if they had 0.15 or more pmol etorphine binding/mg protein or low binding with fewer than 0.15 [6]. High-to-moderate binding loci microinjected with naloxone in this study were: frontal cortex, medial nucleus of amygdala, central nucleus of amygdala, cortical nucleus of amygdala, basal nucleus of amygdala, lateral nucleus of amygdala, anterior area of amygdala, parafascicular nucleus of thalamus, dorsomedial nucleus of thalamus, paraventricular nucleus of thalamus, posterior nucleus of thalamus, rhomboid nucleus of thalamus, ventrolateral nucleus of thalamus, lateral habenula, dorsomedial nucleus of hypothalamus, posterior nucleus of hypothalamus, superior colliculus, and periaqueductal gray. Low binding loci microinjected in the present study were: pyriform cortex, hippocampus, corpus callosum, fasciculus retroflexus, medial lemniscus, internal capsule, stria medullaris, mammillary peduncle, brachium of superior colliculus and central tegmental tract [13].

†Numbers in parentheses refer to number of rats which received a brain microinjection. Each rat received only one brain injection.

Mean=mean number of seconds spent immobile on bar test after a brain microinjection. Reversal=number of rats in each group spending ≤15 sec immobile on bar test expressed as a percent.

present study was undertaken to determine if a relationship exists between the ability of naloxone microinjections to reverse morphine catalepsy and the density of opiate binding within various brain loci. Secondly, we wished to determine if a relationship exists between the naloxone-induced catalepsy reversal and the relative distance of sensitive brain loci from the ventricular core of the brain. The protocol involved a dose-response analysis of the brain loci in which naloxone microinjections reversed the catalepsy produced by intraperitoneal morphine administration.

METHOD

Experimentally naive male Long Evans rats were stereotaxically implanted with one 23 gauge guide cannula [23,24]. Following a two week recovery from surgery, animals were injected intraperitoneally with 80 mg/kg morphine sulfate in distilled water. Previous research has demonstrated that this dose of morphine is nontoxic and produces a catalepsy sufficiently robust to fulfill the requirements of this study [20,24]. Thirty minutes after the peripheral morphine injection, each rat was tested for the presence of a cataleptic response by placing both its forepaws on a horizontal bar 10 cm high and recording the number of seconds the animals maintained this position [2]. Characteristically, an adult rat will maintain a relatively immobile position for many seconds or minutes dependent on the dose of morphine administered, whereas saline injected rats tend to dismount from the bar immediately. Since repeated behavior testing is associated with an increasing cataleptic score [16] each rat was given only a single bar test after morphine administration. Furthermore, a criterion of complete lack of forepaw movement was imposed in order to insure that only rats showing a vigorous response to the narcotic received brain microinjections [3].

Animals keeping both front limbs in position on the bar for 30 seconds received a 1 mcl injection of one of three doses of naloxone hydrochloride in sterile water or sterile isotonic saline. Solutions were administered by means of a 30 gauge Hamilton needle precut to the desired length for each rat and attached to a Hamilton microsyringe. Once the brain injection had been given, the solution was allowed 15 seconds to diffuse away from the needle before the blocking wire was replaced in the implant and the animal returned to its cage. All animals received only one peripheral morphine injection and one brain injection of either naloxone or saline. Five minutes after the chemical brain microinjection the bar test for catalepsy was repeated since pilot studies had shown that reversals generally occurred by 2 minutes postinjection and persisted for 15 minutes. The second bar test was further categorized as a reversal of morphine catalepsy if the animal kept both front limbs immobile on the bar for 15 seconds or less, or as a failure to reverse the catalepsy if the animal maintained its position on the bar for 30 seconds [24]. The

TABLE 2
NALOXONE REVERSAL OF MORPHINE CATALEPSY

Naloxone Microinjection Dose (m cg)	Binding Density* High R	High NR	Low R	Low NR
Saline	0	12	0	6
	R	NR	R	NR
0.01	10	19	0	14
	R	NR	R	NR
0.1	10	19	0	14
	R	NR	R	NR
100	29	4	0	4

*Sites of brain microinjection were classified as high or low density as in Table 1.

R=reversal: bar test score of ≤15 sec after microinjection; NR=nonreversal: bar test score of >15 sec after microinjection.

Number of animals in each group showing reversal or nonreversal of the morphine catalepsy after microinjection.

tests were done blind with respect to site of microinjections. This protocol resulted in data from 141 animals: 123 microinjected with naloxone in doses ranging from 0.01 to 100 mcg; and 18 animals microinjected with isotonic saline. Histological verification of brain microinjection sites was obtained blind by microscopic inspection of thionin-stained 40 micron sections to the nearest 0.25 mm via Pellegrino *et al.* [13]. Prior to statistical analysis the data were grouped according to three criteria: dose of naloxone administered, density of opiate binding for the brain microinjection sites, and proximity of microinjection sites to a ventricle. Sites were classed as having high-to-moderate or low opiate binding density as described in Table 1.

RESULTS

Table 1 presents the mean bar test scores and the probability of reversal of the cataleptic response following microinjection (of saline or naloxone) for brain loci classed as having high or low opiate binding density. Table 2 shows the results with the data collapsed across brain loci. Scores represent the numbers of rats in each group showing reversal (bar test score ≤15 sec after microinjection) or nonreversal (bar test score >15 sec after microinjection) in each group. Overall, 49 out of 91 animals microinjected with naloxone in brain sites having high density of opiate binding showed reversal of the cataleptic response. A chi-square test indicated that this effect of naloxone in the high density sites was significant, $\chi^2(1)=10.75$, $p<0.01$. Across all six regions 20 out of 33 microinjections using 100 mcg naloxone produced reversals while 10 out of 29 microinjections each using 0.1 or 0.01 mcg naloxone resulted in catalepsy reversal. Chi-square analysis showed that the dose effect of naloxone for the high density sites was significant, $\chi^2(2)=23.8$, $p<0.01$. Interestingly, there were no reversals out of 38 naloxone microinjections in brain sites having low density of opiate binding in spite of the fact that some of these sites were only 0.25 mm away from high density sites found effective in other animals. Furthermore, there were no reversals in either high or low density loci following saline microinjections in 18 animals. Taken together, these data suggest that drug used in microinjection (naloxone or saline), naloxone dose, and binding density of microinjection locus may all play a potentially important role in mediating the observed reversal of the cataleptic response. To provide more information about these possibilities additional analyses were carried out on the data.

The relationship between catalepsy reversal and naloxone dose was examined more closely for the high density sites by comparing data for each naloxone dose with the control. Chi-square tests indicated that all doses of the opiate antagonist resulted in significant reversals: 0.01 mcg naloxone; $\chi^2(1)=5.38$, $p<0.025$; 0.1 mcg naloxone, $\chi^2(1)=5.38$, $p<0.025$; 100 mcg naloxone, $\chi^2(1)=28.5$, $p<0.01$. Also, the highest dose of naloxone was found to produce significantly more reversals than either of the two lower doses, $\chi^2(1)=18.6$, $p<0.01$.

In order to determine in a preliminary way the contribution to catalepsy reversal by components of the opiate system which comprised periventricular (central grey, thalamus and hypothalamus) structures chi-square tests were carried out for the high density sites by comparing data for each naloxone dose with the control. Chi-square analyses indicated that the two higher doses of naloxone resulted in significant reversals in the three periventricular structures: 0.01 mcg naloxone, $\chi^2(1)=3.8$, $p>0.05$; 0.1 mcg naloxone, $\chi^2(1)=5.14$, $p<0.025$; 100 mcg naloxone, $\chi^2(1)=10.6$, $p<0.01$. Similarly, in order to evaluate the contribution to reversal by the three non-periventricular structures (caudate nucleus, amygdala and frontal cortex) similar analyses of the high density sites were carried out. These indicated that only for the highest dose of naloxone did significant reversals occur after microinjection into these structures: 0.01 mcg naloxone, $\chi^2(1)=1.7$, $p>0.05$; 0.1 mcg naloxone, $\chi^2(1)=2.1$, $p>0.05$; 100 mcg naloxone, $\chi^2(1)=19.0$, $p<0.01$. Also, a comparison of the catalepsy reversals at the highest dose of naloxone indicated no differences between periventricular and nonperiventricular structures, $\chi^2(1)=0.1$, $p>0.05$, while a comparison at the 0.1 mcg dose indicated a greater sensitivity to naloxone for periventricular structures, $\chi^2(1)=5.7$, $p<0.025$.

DISCUSSION

It is apparent from Tables 1 and 2 that paleospinothalamic, limbic system and basal ganglia sites for the reversal of morphine catalepsy by central naloxone injection form a system which conforms to the location of sites with high opiate binding. The distribution of enkephalin, also generally parallels the neutroanatomical regions implicated in the cataleptic response [18]. Recent evidence suggests that the opiate binding system may mediate the actions of enkephalin [7].

The extension of sites mediating narcotic drug actions from the paleospinothalamic pain system [12] to parts of the limbic system which are involved in motivation and emotional affect [6,19] and parts of the basal ganglia which modulate motor functions [21] is not surprising. It has long been thought that much of the analgesic action of the narcotics results from an effect on the patient's motivations and emotions, while pain responsiveness is usually revealed by enhanced functioning of the motor system [19].

The extent to which different components of this receptor binding system have different roles in narcotic-induced catalepsy is unclear. Whether this system-wide involvement also applies to other narcotic drug actions such as analgesia, euphoria, tolerance, and dependence is unclear as well.

However, the type of neuroanatomical and dose-response analysis represented by this study would seem to hold some promise of contributing to the answers to these questions. This approach should also provide information about the separability of narcotic drug actions and the likelihood of developing improved drugs of the narcotic analgesic type. For example, if neuroanatomical dose-response maps for rigidity, catalepsy, analgesia, reinforcement enhancement, and dependence showed considerable overlap, it would seem unlikely that these actions were separable. If, on the other hand, differentiable sites mediated these various actions, then pharmacological separation of effects would seem a more obtainable goal.

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