Pharmacology Biochemistry & Behavior, Vol 29, pp 573–580 © Pergamon Press plc, 1988 Printed in the U S A 0091-3057/88 $3 00 + 00

Localization of Brainstem Sites Which Mediate Alfentanil-Induced Muscle Rigidity in the Rat

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Received 11 May 1987

WEINGER, M B, E J CLINE, N T SMITH, T A BLASCO AND G F KOOB *Localization of brainstem sites which mediate alfentanil-induced muscle rigidity in the rat* PHARMACOL BIOCHEM BEHAV **29**(3) 573–580, 1988 —Previous work has demonstrated that direct injections of methylnaloxonium (MN), a relatively lipophobic quaternary opiate antagonist, in the area of the nucleus raphe pontis (RPn) significantly attenuated alfentanil-induced rigidity It was hypothesized that other hindbrain sites, particularly the other raphe nuclei, might play a role in this rigidity Therefore, a study was performed in which 57 rats, divided into four groups, were implanted with chronic guide cannulae directed at brain sites anterior, lateral, or posterior to the RPn After each animal was pretreated with intracerebral injections of MN, alfentanil (0 5 mg/kg) was administered subcutaneously Electromyographic activity was recorded from the gastrocnemius muscle as a measure of hindlimb rigidity Each animal was subsequently injected at 4 to 5 day intervals with MN two addtional times at sites 1 and 2 mm deeper, respectively, than the initial injection Data were thus obtained on animals treated with either MN or saline at 3 successive histologically identified sites which were either anterior, lateral or posterior to the RPn The administration of MN into two specific sites in the region just lateral to the nucleus raphe pontis significantly [F(1,38)=18 68 and 5 02 respectively, $p<0$ 05] reversed the rigidity produced by systemic alfentanil administration There was a weak effect of MN injections anterior to the RPn but this could not be localized to any one site These results suggest that discrete brainstem regions involved in opiate action can be sensitively and selectively identified by direct intracranial injections of a lipophobic opiate antagonist Based on the results of this study and previous work, it appears that a specific hindbrain region, known to contain serotonergic pathways, mediates opiate-induced muscle rigidity in the rat

Opiates Alfentanil Methylnaloxonium Muscle rigidity Catatonia Electromyography Raphe nuclei Nucleus raphe pontis Reticular formation

THE neuroanatomic substrates of opiate-induced muscle rigidity have yet to be fully elucidated Besides being a fascinating physiological phenomenon, muscle rigidity is also a clinically significant side-effect of high-dose narcotic anesthesia [5] An improved understanding of the mechanisms and sites of action of opiate-induced rigidity may give insight into other causes of muscle rigidity as well as being an important advance in opiate research Previous investigators have emphasized the role of the basal ganglia [25] More recently it was demonstrated that direct injections of methylnaloxonium (MN), a quaternary opiate antagonist, in the area of the nucleus raphe pontis (RPn) were significantly more effective at preventing alfentanil-induced rigidity than were injections into the caudate nucleus [7] Alfentanil is a highly potent, short-acting fentanyl analog [18] that produces the rapid onset of profound muscle rigidity in both rats [7] and humans [5]

The RPn (see Table 1 for full list of abbreviations) is one of a number of interconnected midline (raphe) nuclei in the rodent brainstem reticular formation that have been implicated in mediating a variety of physiological processes, including motor behavior and analgesia The raphe nuclei are also the major source of serotonergic neurons in both the rat and human brains [34] Serotonergic systems appear to play an important role in rodent motor behaviors including those affected by opiate administration For instance, Costall and

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TABLE 1

ABBREVIATIONS

ALF	Alfentanil Hydrochloride	PAG	Periaqueductal Gray
CN	Caudate Nucleus	RMg	Nucleus Raphe Magnus
CSC	Commissure of the Superior Colliculus	RMS	Root-mean-squared
DR	Dorsal Raphe Nucleus	ROb	Nucleus Raphe Obscurus
DTg	Dorsal Tegmentum	RPd	Nucleus Raphe Pallidus
EMB	Explosive Motor Behavior	RPn	Nucleus Raphe Pontis
EMG	Electromyography	SAL	Saline
GABA	Gamma Amino Butyric Acid	sc	Subcutaneous
ICV	Intracerebroventricular	SC	Superior Colliculus
MN	Methylnaloxonium Hydrochloride	SNC	Substantia Nigra Pars Compacta
MnR	Median Raphe Nucleus	SNR	Substantia Nigra Pars Reticulata
MS	Morphine Sulfate	St	Striatum
NAc	Nucleus Accumbens	VMT	Ventromedial Thalamus
NRTP	Nucleus Reticularis Tegmenti Pontis	5-HT	Serotonin

coworkers [19] showed that the administration of the serotonin antagonist cyproheptadine diminished the catatonia produced by direct injections of morphine into the rat brain. Fenfluramine, an indirect serotonin agonist, potentiated the catatonia produced by the injection of morphine in the region of the RPn [12] In addition, it has been shown that systemic pretreatment with ketanserin, a relatively selective serotonin receptor antagonist [28], prevents alfentanil-induced muscle rigidity [38]

The serotonin neurons within the RPn project primarily to the cerebellum, although there are also some ascending and spinal efferents [6,11] Most of the descending serotonergic projections originate in the medullary raphe nuclei (magnus, obscurus, and pallidus) [34] and coexist with descending peptidergic and cholinergic neurons [9] However, the dorsal and the median raphe nuclei send ascending serotonergic pathways to the basal ganglia and the limbic system, respectively [8,24]. Pharmacological blockade of a variety of common neurotransmitter systems, including serotonin, within the rodent lumbosacral spinal cord failed to antagonize alfentanil-induced muscle rigidity [39] It thus appears that any serotonergic involvement in opiate-induced rigidity is due to supraspinal pathways

Other brainstem sites, particularly the other raphe nuclei, may play a role in opiate-induced muscle rigidity This study was designed to further characterize the role of brainstem structures in mediating alfentanil rigidity in the rat by injecting methylnaloxonium at several different depths anterior, lateral, and posterior to the RPn

METHOD

Subjects

The subjects were 57 albino Wistar rats (Charles River Laboratories, Wilmington, MA) weighing 200 to 260 g at the time of surgery. They were housed 3–4 per cage with food and water available continuously in a temperature-controlled room. A 12-hour light, 12-hour dark schedule was maintained with the light period between 7 a m. and 7 p m. Physiological recording was always done in the morning from 8 a.m to 12 noon. Rats were handled prior to the experimental days to minimize potential effects of stress on the results These experiments were conducted over the course of a 6 month period from January to July of 1986 The conduct of this study met the guidelines established by our institutional animal care committee

Drugs

Drugs used were methylnaloxonium hydrochloride (courtesy of Dr J deGraaf, Organon Pharmaceutica, Oss, The Netherlands), sodium pentobarbital (Abbott Laboratories, North Chicago, IL), and alfentanil hydrochloride (Janssen Pharmaceutica, Piscataway, NJ) All drugs, obtained as powders, were dissolved in 0 9% physiological saline and injected in a volume of 1 ml/kg The alfentanil dose used throughout the study was 0 5 mg/kg because this was the lowest dose which yielded consistent rigidity in previous studies [2, 7, 38]

Surgical Preparation

All animals were surgically implanted with permanent guide cannulae for intracerebral injections To accomplish this, the animals were anesthetized with pentobarbital (50 mg/kg IP) and then positioned in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) with the incisor bar set at 5 0 mm above the interaural line The skull surface was exposed, a burr hole was made, and a 10 mm, 23-gauge chronic guide cannula was then stereotaxically lowered to a location 4 mm above the coordinates for the last of the three injection sites

Four experimental groups were studied The *Bilateral* group (n=17) were implanted with two cannulae using the coordinates AP −1 0 mm, Lateral ±1 0 mm, and DV −5 5 mm (from skull surface) [32] The other groups had single midline cannulae implanted using the coordinates. *Anterior* (n=13), AP 0 0, L 0 0, DV −5 5, *Posterior* (n=12), AP −2.0, L 0 0, DV −5 5, and *Posterior Extension* (n=15), AP −2 0, L 0 0, and DV −7 5 All guide cannulae were fixed to the skull surface with stainless steel screws and dental cement (Sun-Schein® visible light curing restorative) Ten mm obturator stylets remained in place except during drug injections Following surgery, the animals were allowed 5–6 days to recover before conducting electromyographic studies

Experimental Protocol

As described above, the animals were divided into four experimental groups based on the anatomical location of the chronic guide cannulae. *Anterior, Bilateral, Posterior,* and *Posterior Extension* For the first study in each animal, 12 mm injectors were inserted into the guide cannula and 1 μl of saline (SAL controls) or 0 125 μg (total dose) MN in 1 μl of saline was infused using an automated microliter syringe

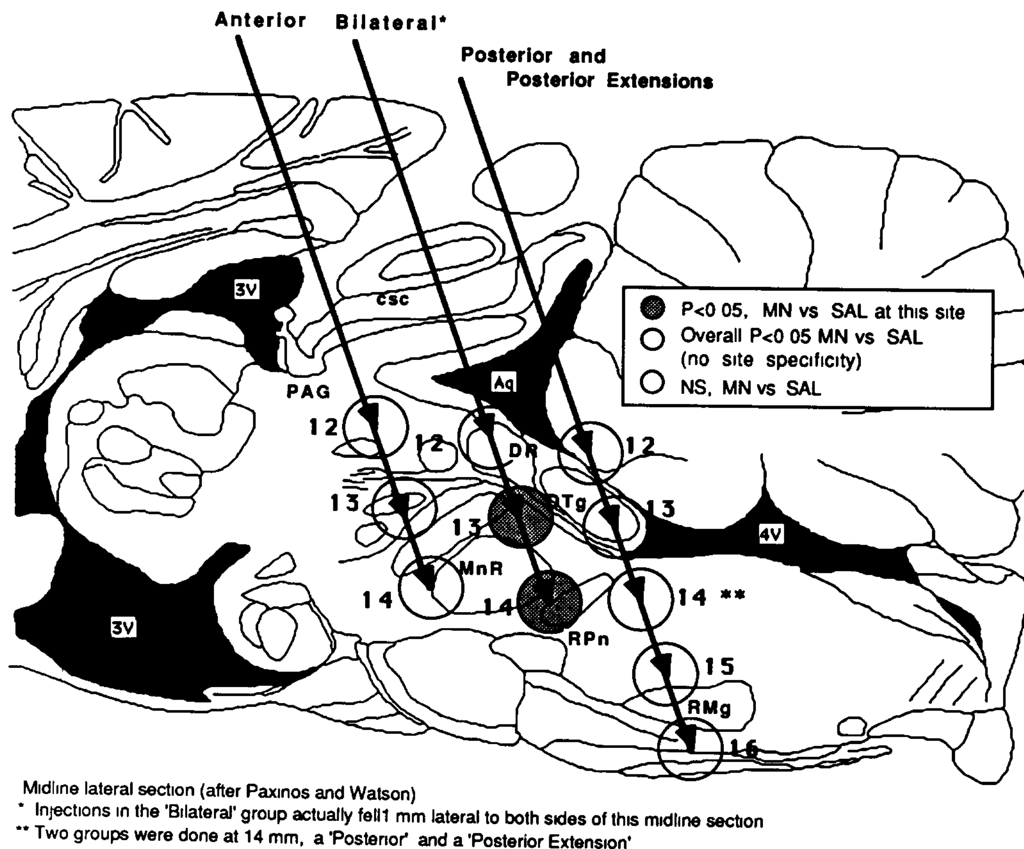

FIG 1 The sequence and the approximate location of each injection site is depicted on this figure of a median sagittal section of a rat brain Note [*] that *Bilateral* injection sites actually lie 1 mm on both sides of the displayed midline section and therefore their histological location is slightly distorted in this figure Refer to Fig 3 for a more accurate portrayal of the anatomical location of the *Bilateral* injection sites Also note [**] that two groups were studied at 14 mm posterior to the RPn, the last injection site in the *Posterior* group and the first injection site in the *Posterior Extension* group The only significant drug-site interactions occurred at the 13 mm and 14 mm sites in the *Bilateral* group (lateral to the RPn) At these two sites, MN injections significantly ($p<0$ 05) attenuated alfentanil-induced rigidity (dark shading) compared with saline

pump (Harvard Bioscience 55-3206) over 3 minutes Animals in the *Bilateral* group received 0 0625 μg of MN (in 1 μl) via each cannula The injectors were not removed for 2 minutes after the intracranial injection to preclude the back-diffusion of the drug up the cannula tract. This procedure was repeated on each animal at 4 or 5 day intervals with 13 and then 14 mm injection depths using a "within subjects" experimental design with parallel-tested separate control groups (see Fig 1). Each animal therefore received injections of either MN or SAL, once at each of three successive depths, over a 12–15 day period

Generally, 4 rats were studied each day with equal numbers of MN animals and their corresponding SAL control animals Thus, within each group, the paired MN and SAL animals were exposed to nearly identical experimental conditions at each injection depth

After each intracerebral SAL or MN injection, the animals were immediately placed in barred cylindrical holding cages which allowed free movement of the extremities as well as easy access to injection and recording sites (Fig 2) Two monopolar (10 mm × 100 μm diameter) platinum recording electrodes (Grass E2) were placed percutaneously into the left gastrocnemius muscle, while a third (ground) electrode was inserted subcutaneously into the right hindlimb. Leads were secured with cellophane tape in a manner that allowed unimpeded joint mobility. Two caged animals at a time were placed inside a sound-proof box (Coulbourn Instrument Company) A cardboard partition was put between the cages and an electric fan was run continuously to provide white noise EMG activity was then assessed for a 15-minute baseline period, with readings recorded at 5, 10, and 15 minutes after intracranial injection

Actual muscle potentials were differentially amplified 200 times and band-pass filtered from 10 Hz to 3 kHz (Grass P511K) The resulting signal, viewed on an oscilloscope (Tektronics 7633), was then converted with a root-mean-squared (RMS) voltage rectifier ($t_{1/2}$ = 3 sec) to produce a time-varying analog deflection on a 200 mV meter (Triplet 820-M) from which data were obtained Full-scale deflection of the meter corresponded to 200 μV of EMG activity

At the end of the 15-minute baseline period, each rat was injected *in situ* subcutaneously with alfentanil Readings were obtained at 1 and 5 minutes post-injection, and then at 5 minute intervals throughout the remainder of the 60-minute observation period. During data collection, care was taken to eliminate the effects of transient movement artifacts, thereby permitting an assessment of tonic rather than phasic muscle activity

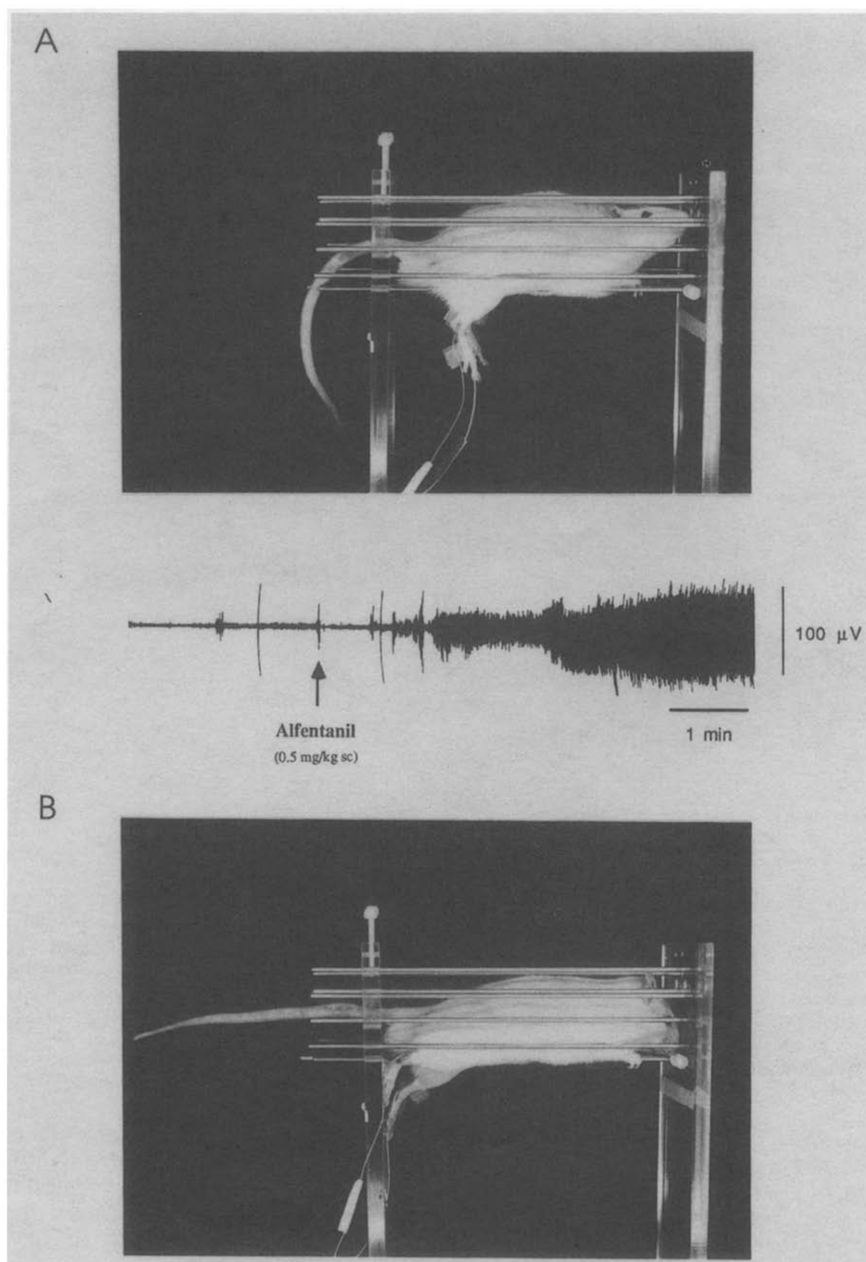

FIG 2 A rat, placed inside a cylindrical holding cage, both before (A) and after (B) alfentanil injection A representative EMG tracing demonstrates the effects of alfentanil (0 5 mg/kg SC) on EMG activity (at arrow)

Histological Verification

At the end of the study each animal was deeply anesthetized with pentobarbital (100 mg/kg IP) and then perfused via a needle inserted into the left cardiac ventricle with 0.9% saline followed by 10% formalin The brain was removed intact and preserved in 10% formalin At least two days later, the brain was sectioned using a microtome The fifty-micron sections were then mounted and Nissl stained for verification of injection sites

Data Analysis

Data from each animal's 60-minute observation period were normalized by their respective average baseline EMG reading on that test day This was accomplished by obtaining the average of the three baseline (5-min) readings for each animal and dividing this value into all subsequent values obtained during the 60-minute observation period Animals with average baseline values of less than 2 μV or more than 40 μV were excluded from the study The mean area under the normalized EMG curve for each treatment observation was then calculated Statistical differences between MN and SAL pretreatment from each group (*Bilateral, Anterior, Posterior,* and *Posterior Extension*) were determined using two-way Analyses of Variance (ANOVA) with repeated measures on one factor (depth of injection) followed by

TABLE 2

Injection Site	Weight (g)		Baseline EMG (μV)	
	Saline*	MN	Saline*	MN
Anterior				
12 mm	285 ± 8†	283 ± 12†	8.2 ± 1.3	12.0 ± 3.4
13 mm	316 ± 7	326 ± 9	6.0 ± 0.6	8.6 ± 1.1
14 mm	334 ± 4	349 ± 9	8.2 ± 1.2	7.6 ± 1.0
Bilateral				
12 mm	258 ± 7†	263 ± 7†	12.4 ± 2.0	18.0 ± 5.0
13 mm	278 ± 7	291 ± 7	11.6 ± 2.6	14.6 ± 3.8
14 mm	309 ± 7	314 ± 4	11.6 ± 2.6	10.6 ± 1.8
Posterior				
12 mm	281 ± 15†	287 ± 12†	7.4 ± 1.2	8.6 ± 1.6
13 mm	299 ± 6	304 ± 6	8.0 ± 2.2	9.0 ± 1.3
14 mm	315 ± 14	330 ± 5	7.0 ± 2.0	6.4 ± 1.0
Posterior Extensions				
14 mm	236 ± 5†	254 ± 8†	10.0 ± 1.2	11.2 ± 1.8
15 mm	264 ± 9	270 ± 9	7.4 ± 0.6	8.6 ± 1.0
16 mm	293 ± 10	297 ± 10	8.4 ± 1.0	9.4 ± 1.4

*Expressed as mean ± S.E.M.
†$p<0.05$ between increasing injection depths within one experimental group (by ANOVA).

TABLE 3

Injection Site	Depth	Area Under EMG Curve	
		Saline*	Methylnaloxonium
Anterior	12 mm	51.1 ± 8.6	23.6 ± 3.5†
	13 mm	40.1 ± 5.9	25.6 ± 6.4
	14 mm	42.7 ± 6.6	34.4 ± 6.3
Bilateral	12 mm	36.3 ± 2.7	28.7 ± 4.9
	13 mm	47.7 ± 5.9	19.3 ± 1.7‡
	14 mm	36.4 ± 5.5	21.7 ± 3.2§
Posterior	12 mm	50.8 ± 12.8	36.0 ± 9.7
	13 mm	43.9 ± 9.1	27.8 ± 6.7
	14 mm	54.6 ± 16.6	42.9 ± 10.2
Posterior Extensions	14 mm	45.1 ± 6.9	39.1 ± 5.3
	15 mm	56.2 ± 9.1	34.8 ± 10.4
	16 mm	45.5 ± 4.2	32.9 ± 5.2

*Expressed as mean ± S.E.M. Data analyzed by two-way ANOVA followed by Newman-Keuls.
†$p<0.05$, overall simple main effect of MN vs. SAL without drug-site interaction.
‡$p<0.001$ between saline and MN groups at this site.
§$p<0.05$ between saline and MN groups at this site.

Newman-Keuls *a posteriori* tests. Baseline EMG activity and mean animal weights between treatment groups and injection depths in each experimental group were analyzed in the same manner. Data were expressed as mean±S.E.M. and a p value of less than 0.05 was considered statistically significant.

RESULTS

The animals' weight generally increased over the course of each experiment since 8 to 10 days elapsed between the first and the last intracerebral injections (Table 2). There was, however, no statistically significant difference in mean weight between MN and SAL groups at any injection depth in any experimental group. Baseline EMG values were also not statistically different between any experimental group.

Concurrently, a separate group of animals chronically implanted with cannulae at the *Anterior* (n=5) and *Posterior* (n=6) sites was studied to test for tolerance. These animals were given ALF without any prior intracerebral injections. They showed EMG activity (overall mean: 38.9±9.6) which was statistically indistinguishable from animals whose first ALF injection was preceded by an intracerebral injection of saline.

Only within the *Bilateral* group was there a significant drug-depth interaction, $F(2,30)=3.67$, $p<0.05$. Further analysis revealed that only MN injections at both the 13 mm, $F(1,38)=18.68$, $p<0.001$, and the 14 mm, $F(1,38)=5.02$, $p<0.05$, depth significantly reduced rigidity compared with SAL controls. Note that MN injections at these two sites resulted in the lowest average rigidity scores of any group. On histological examination, these injection sites were just lateral to, but not within, the RPn. However, because many of the *Bilateral* cannulations were not precisely centered with respect to the cerebral midline, one of the two lateral injection cannulae was generally slightly closer than 1 mm to the RPn.

While overall within the *Anterior* group, injections of MN (at all sites combined) significantly attenuated rigidity compared with SAL injections, $F(1,11)=5.0$, $p<0.05$, the drug-depth interaction failed to attain statistical significance, $F(2,22)=2.06$, $p>0.15$. Thus, while a weak simple main effect was obtained, it was insufficient to identify a specific site of MN action in the *Anterior* group despite the fact that the greatest mean experimental difference between MN and SAL occurred at the 12 mm injection depth in this group. Histologically, the 12 mm *Anterior* injection site was in the region of the periaqueductal gray (not too distant from the deep layers of the superior colliculus and the dorsal raphe nucleus) while the 14 mm injection site appeared to be in the region of the median raphe nucleus (see Fig. 1).

There were no significant differences between MN and SAL injections in the *Posterior* or the *Posterior Extensions* groups ($F<1.0$). Histological examination of the brains revealed that the lesions produced by the final injection in every animal fell within a circle with a radius of approximately 1 mm.

Figure 3 depicts the results of detailed histological examination of the sites of MN injection in the *Bilateral* group. Using a criteria of at least a 40% reduction in EMG activity compared with matched SAL controls, one can see that the more ventral injection sites lateral to the raphe nuclei within the pons appear to be the most sensitive sites (solid circles in the figure) for reversal of alfentanil rigidity by prior MN injection. Note that for visualization, the circles representing injection sites are depicted somewhat smaller than the areas of actual tissue damage.

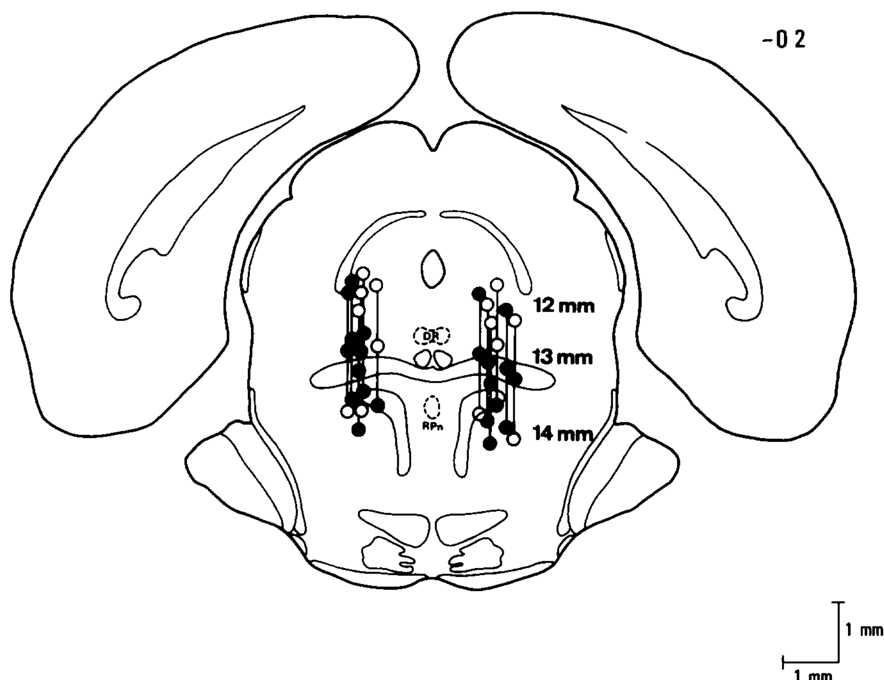

FIG 3 The results of detailed histological examination of the sites of MN injection in 6 animals from the *Bilateral* group Two brains were inadvertently lost prior to detailed examination and are not included in this figure although approximate injection location had been confirmed during initial sectioning The 12 and 13 mm injection sites were estimated by measuring back from the identified 14 mm site on this frontal section (after Pellegrino, Pellegrino and Cushman) Note that for visualization, the dots representing injection sites are depicted somewhat smaller than the areas of actual tissue damage Using a criteria of at least a 40% reduction in EMG activity compared with matched SAL controls, one can see that the more ventral injection sites (13 and 14 mm) lateral to the raphe nuclei within the pons appear to be the most sensitive sites (solid circles) for reversal of alfentanil rigidity by prior MN injection Open circles depict MN injection sites which produced less than a 40% decrease in rigidity compared with SAL DR dorsal raphe nucleus, RPn raphe pontis nucleus

DISCUSSION

Subcutaneous injections of alfentanil produce the rapid onset (1–2 minutes) of an intense muscle rigidity which generally persists for 30 to 45 minutes This rigidity can be readily reversed by the systemic administration of the opiate antagonist naloxone [25] In addition to exhibiting rigidity, animals that receive ALF are akinetic, lose their righting reflex, and appear to be in a trance-like state

The present technique for studying the sites of action of opiate-induced muscle rigidity in the rodent brain is different from that of previous investigators [19, 22, 25, 26] Animals are pretreated with direct intracerebral injections of the hydrophilic opiate antagonist methylnaloxonium and then, after the insertion of EMG leads, are injected subcutaneously with the potent opiate agonist, alfentanil There are two distinct advantages of this protocol, one relating to the use of MN, and the other to the use of ALF First, because MN is hydrophilic, the drug appears to remain relatively localized to the intracranial site of injection with less diffusion or systemic absorption than that seen with more lipophilic agents like naloxone [13] MN has been shown to be an effective antagonist at the opiate receptor [1] Secondly, because ALF has a very rapid onset and a relatively short duration of action [18], baseline (pre-ALF) EMG activity can be recorded and then, almost immediately after ALF injection, a reliable increase in EMG activity occurs which corresponds to profound muscle rigidity

The results of the present study appear to substantiate the sensitivity and selectivity of this experimental technique for investigating the sites of action of opiate-induced muscle rigidity in the rodent brain One cannot, however, rule out the possibility that the effects of centrally administered MN resulted, at least in part, from a diffusion of the drug to nearby brain regions It has been estimated that a 1 μl intracranial injection of any drug, regardless of its hydrophilicity, will spread out in a sphere with an approximate radius of 1 mm [31] This may be of particular concern in the brainstem where there is a concentration of a large number of discrete nuclei, many of which are involved in motor control [10]

It is also possible that the mechanical effects of intracerebral injection at one or several sites produced local trauma which altered the normal pathways regulating motor tone and behavior and thereby potentially affected the animal's response to ALF This possibility was tested by using parallel SAL control groups at each injection site Dunstan *et al* [21], employing extensive intracerebral injections of morphine, were the first to implicate the brainstem reticular formation in opiate-induced rigidity Blasco and coworkers [7] subsequently showed that 0.125 μg of MN injected into the RPn significantly attenuated ALF-induced rigidity In contrast, 2.0 μg of MN injected intraventricularly was necessary to significantly decrease rigidity while MN doses up to 4 0 μg infused into the caudate nucleus had no effect A similar pattern was obtained by assessing ALF-

induced catatonia or total locomotor activity after intracerebral MN injections into the RPn and CN [2].

The results of the present study suggest that very few discrete lower brain regions mediate opiate-induced muscle rigidity. While it is possible that lateral structures at the level of the RPn were responsible for the attenuating effects of MN injections at 13 mm in the *Bilateral* group, the midline RPn may have, in fact, also been affected due to the adjacent injection of MN This could partly be accounted for by imperfect centering of the bilateral cannulae about the midline

Injections at 12 mm in the *Anterior* group were in the region of the periaqueductal gray (PAG) or nearby midbrain structures Previous work has suggested that these regions may be involved in the expression of rigidity [14, 35, 36] MN administered specifically into the deep layers of the superior colliculus significantly reversed ALF-induced rigidity (unpublished). In a recent study by Widdowson [40], direct injections into the PAG of the mu agonist d-ALA^{2}-MePhe^{4}-Gly-ol^{5}-enkephalin or β-endorphin produced significant limb rigidity which could be reversed by ICV naltrexone Pretreatment with 5,7-dihydroxytryptamine prevented the rigidity suggesting that serotonergic pathways play a role within the PAG

Injections at the 14 mm depth in the *Anterior* group were in the region of the median raphe nucleus while those at the 12 mm depth in the *Bilateral* group fell just lateral to the caudal portion of the dorsal raphe nucleus. Further studies must be performed with injections directly and specifically into these more rostral raphe nuclei before any conclusions can be drawn about their role in mediating opiate-induced rigidity

The raphe nuclei are a major source of serotonergic pathways which may play an important role in opiate-induced rigidity and catatonia [3, 12, 19, 39] The dorsal and the median raphe nuclei send ascending serotonergic pathways to the basal ganglia and the limbic system, respectively [8,24] On the other hand, most of the descending serotonergic projections originate in the medullary raphe nuclei, while the serotonin neurons within the RPn project primarily to the cerebellum [6, 11, 34] In contrast to the role of these descending 5-HT pathways in mediating analgesia [29], recent work suggests that descending serotonergic neurons do not play a role in the expression of alfentanil-induced rigidity [39] It thus appears that any serotonergic involvement in opiate-induced rigidity is due to supraspinal pathways

Previous investigators have emphasized the basal ganglia as an important site for opiate-induced rigidity [25] The basal ganglia not only contains high concentrations of opiate receptors [27] but it is crucial to the extrapyramidal control of muscle tone and posture [4] GABAergic pathways within the basal ganglia seem to play an important role in mediating rigidity Muscle hypertonus can be induced by injections of muscimol into the substantia nigra pars compacta [26] or into the ventral aspect of the neostriatum [37] Similarly, rigidity occurs if striatonigral GABAergic transmission is decreased by injection of bicuculline into the substantia nigra pars reticulata [26] The striatonigral tract has been shown to terminate monosynaptically on GABAergic neurons of the substantia nigra pars reticulata which then project to the ventromedial thalamus [33]. GABAergic pathways also project from the nigra to the superior colliculus [20] Both the deep layer of the superior colliculus [22,23] and the ventromedial thalamus [30] appear to be involved in the expression of hindlimb rigidity

Chesire and coworkers have implicated the nucleus reticularis tegmenti pontis (NRTP), a brainstem structure just ventrolateral to the RPn, in rodent motor behavior Lesions of the NRTP or direct injections of opiates or GABA into this region reversed the akinesia produced by systemic morphine or haloperidol [16,17] They hypothesized that systemic opiates produce 'explosive motor behavior' by inactivating an inhibitory pathway in this region and that serotonin plays a role in the activity of the NRTP [15] The stereotaxic coordinates used by Cheshire and co-investigators for the NRTP were only a few millimeters ventral to those used in our *Bilateral* group Because of the close proximity of the NRTP to the RPn in the rodent, one cannot exclude the possibility that some of the effects of MN injection in the RPn (or bilateral to it) on ALF-induced rigidity are due to action on the NRTP On the other hand, it is also possible that some of the effects reported by Chesire and coworkers were due to activity in the RPn rather than the NRTP

In conclusion, injection of the relatively lipophobic opiate antagonist methylnaloxonium into the region of the nucleus raphe pontis, but not in adjacent sites posterior or ventral to this area, significantly reversed the increased hindlimb electromyographic activity produced by the systemic administration of alfentanil Further studies using this technique may permit a detailed mapping of the neural circuitry involved in opiate-induced rigidity These results suggest that specific hindbrain sites, known to contain GABAergic and serotonergic pathways, may play an important role in the muscle rigidity associated with opiate drugs and may also be involved in mediating normal motor behavior

ACKNOWLEDGEMENTS

This work was supported in part by a generous gift from Janssen Pharmaceutica (Piscataway, NJ) and also by Grants from NIDA (01785) and NIAAA (03504)

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