Title: **FURTHER ELUCIDATION OF BRAIN SITES WHICH MEDIATE ALFENTANIL - INDUCED MUSCLE RIGIDITY IN THE RAT.**

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**Introduction** Previous work, seeking to elucidate the neuroanatomical substrates of opiate-induced muscle rigidity, suggests that brain sites in the region of the nucleus raphe pontis (RPn) [1,2] and also in the area of the periaqueductal gray matter (PAG) [3] play a role in this rigidity. Direct injections of methylnaloxonium (MN), a quaternary opiate antagonist, in the area of the RPn were significantly more effective at preventing alfentanil-induced rigidity than were injections into the caudate nucleus [1]. In a follow-up study, the nucleus reticularis pontis [NRTP], just lateral to the RPn, was also shown to play a role in alfentanil (ALF) rigidity [2]. This study demonstrated that intracranial injections of minute amounts (0.125 μg) of MN could sensitively identify brain sites mediating ALF rigidity. However, because multiple brain regions were cannulated in each animal, only the anatomical location of the last injection site could be precisely determined. This precluded definitive conclusions about the role other brainstem regions. The purpose of the present study was to further characterize the neurophysiology of opiate rigidity in the rat by injecting MN or saline into multiple discrete histologically-verified brain sites and analyzing the effects on ALF-induced increases in hindlimb electromyographic (EMG) activity.

**Methods** After Animal Care Committee approval, 138 male Wistar rats (200-300 gm) had 10-mm, 23-gauge guide cannulae stereotaxically implanted (incisor bar 5.0 mm above interaural line) while under pentobarbital anesthesia. Multiple brain sites, many previously implicated in the control of muscle tone, were cannulated using coordinates from Pellegrino et. al. [4]. For each group, after at least 5 days of recuperation, 13 mm injectors were inserted into the guide cannula of each animal and 1 μl of saline (SAL) or 0.125 μg (total dose) MN was infused into the discrete brain sites by Harvard pump over 3 minutes. Pairs of animals were injected in the same site in a "blinded" fashion; one rat receiving SAL and one MN. Both animals were then placed in cages and muscle rigidity was measured by EMG recordings from the left gastrocnemius muscle as described previously [1,2]. After 15 minutes of baseline measurements, the rats were injected with ALF (0.5 mg/kg s.c.) and data were collected at 5 minute intervals for 60 minutes. At the end of the study each animal was deeply anesthetized, perfused with intracardiac formalin, and its brain was removed intact. Fifty micron sections were mounted and Nissl stained for verification of injection sites. EMG values for each rat were normalized for that animal's baseline readings. For each group, the average of the area under the normalized EMG voltage-versus-time curve was calculated. Statistical differences between associated MN and SAL groups were assessed using one-way ANOVA. For comparisons between groups, the average values from MN injected animals were expressed as percent of control rigidity (average values in the SAL animals in that group).

**Results** For all experimental groups, the two treatment groups (MN vs. SAL) did not differ in either mean weight or baseline EMG values. MN injections in the pontine reticular formation and in the area of ventral PAG significantly attenuated ALF rigidity (see **figure**). In confirmation of previous work, both the RPn and the NRTP were sensitive sites for the reversal of rigidity. Within the midbrain, MN selectively reversed rigidity when injected into the dorsal PAG (dPAG) and the adjacent deep layers of the superior colliculus (dSC) but not into the contiguous ventral PAG (vPAG) or the superficial superior colliculus (sSC). There was no significant effect on ALF rigidity of MN injections into the substania nigra reticulata (SNR), nucleus raphe magnus (RMg), dorsal raphe nucleus (DR), nucleus accumbens (NAc), or the region of the decussation of the dorsal tegmentum (dcDTg).

**Discussion.** This study provides strong evidence that only a few discrete brain regions mediate ALF-induced muscle rigidity in the rat. The results confirm earlier work from this laboratory that the region of the RPn/NRTP is a crucial neuroanatomical substrate of opiate-induced rigidity. Using selective cannulation and careful neuroanatomical verification, this study specifically identifies functional brain regions which do and do not mediate opiate rigidity. Interestingly, in contrast to the known role of the other raphe nuclei in opiate analgesia, the other raphe nuclei do not appear to play a significant role in muscle rigidity. The region of the RPn/NRTP contains important serotonergic and GABAergic projections to the spinal cord and cerebellum. Likewise, the PAG contains GABAergic, opioidergic, and adrenergic systems while the superior colliculus is a GABAergic relay station between the basal ganglia and the brainstem reticular formation. Each of these neurotransmitters may play a role in opiate rigidity. Continued work on opiate-induced rigidity will lead to an improved understanding of the neurochemical basis of opiate effects and also will increase our knowledge regarding the mechanisms of other types of centrally-mediated rigidity states. The results of this study have helped direct the develepment of improved clinical regimens for the prevention and treatment of opiate-induced muscle rigidity.

**References.**

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