Title: LOCALIZATION OF BRAIN SITES WHICH MAY PLAY A ROLE IN ALFENTANIL-INDUCED MUSCLE RIGIDITY IN THE RAT

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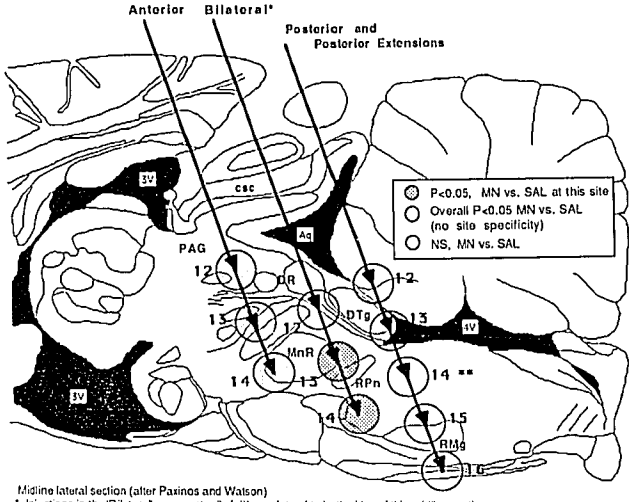
Introduction. The neuroanatomic substrates of opiate-induced muscle rigidity have yet to be fully elucidated. While previous investigators have emphasized the role of the basal ganglia [1], others have demonstrated that direct injections of methylnaloxonium (MN), a quaterary opiate antagonist, in the area of the nucleus raphe pontis (RPn) were significantly more effective at preventing alfentanil-induced rigidity than were injections into the caudate nucleus [2]. Like the other raphe nuclei, the RPn is known to contain serotonergic cell bodies. Pretreatment with ketanserin, a relatively selective serotonin receptor antagonist, prevents alfentanil-induced muscle rigidity [3]. It is possible that other brainstem sites, particularly the other raphe nuclei, play a role in this rigidity. This study was designed to further characterize the role of brainstem structures in mediating alfentanil rigidity in the rat by injecting MN at several different depths anterior, lateral, and posterior to the RPn.

Methods. 60 male Wistar rats (200-300 gm), divided into four groups, had 10 mm, 23-gauge chronic guide cannulas stereotaxically implanted (incisor bar 5.0 mm above interaural line) under pentobarbital anesthesia. The *Bilateral* group (n=18) were cannulated using the coordinates: AP -1.0 mm, Lateral ±1.0 mm, and DV -5.5 mm. The other groups included: *Anterior* (n=13) [AP 0.0, L 0.0, DV -5.5], *Posterior* (n=13) [AP -2.0, L 0.0, DV -5.5], and *Posterior Extensions* (n=16) [AP -2.0, L 0.0, and DV -7.5]. After at least 5 days of recuperation, 12 mm injectors were inserted into the guide cannula of each animal and 1 µl of saline (controls) or 0.125 µg (total dose) MN was infused by Harvard pump over 3 minutes. The animals were then placed in cages and muscle rigidity was measured by electromyographic (EMG) recordings from the left gastrocnemius muscle as described previously [2,3]. After 15 minutes of baseline measurements, the rats were injected subcutaneously with alfentanil (0.5 mg/kg) and data were collected at 5 minute intervals for 60 minutes. This procedure was repeated twice, at 3-5 day intervals with 13 and 14 mm injectors respectively, using a "within subjects" experimental design (**Figure**). At the end of the study each animal was deeply anesthetized, perfused with intracardiac formalin, and had its brain removed intact. Brains were sectioned (50 µm), mounted, and Nissl stained for verification of injection sites. The 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 saline treatments in each experimental group were assessed using a two-way analysis of variance (ANOVA) followed by a Newman-Keuls *a posterori* test. A P value of 0.05 was considered statistically significant.



Midline lateral section (after Paxinos and Watson)
* Injections in the 'Bilateral' group actually fell1 mm lateral to both sides of this midline section.
** Two groups were done at 14 mm; a 'Posterior' and a 'Posterior Extension'

Results. MN injections 1 mm bilateral to the region of the RPn significantly reduced rigidity at both the 13 mm ($F[1,38]=18.68$, $P<0.001$, see **table**) and the 14 mm ($F[1,38]=5.02$, $P<0.05$) injection depths. While overall, within the *Anterior* group, MN injections (at all sites) significantly attenuated rigidity compared with saline ($F[1,11]=5.0$, $p<0.05$), the drug-site interaction was not significant ($F[2,22]=2.06$, $p>0.15$). There were no significant differences between MN and saline pretreatment in the *Posterior* or the *Posterior Extensions* groups.

Discussion. The results of this study confirm that discrete brainstem regions mediate opiate-induced muscle rigidity. Lateral structures at the level of the RPn, such as the nucleus reticularis tegmenti pontis (NRTP), may have been responsible for the attenuating effects of MN injections at 13 and 14 mm in the *Bilateral* group (see **figure**). Both the RPn and the NRTP contain GABAergic as well as serotonergic pathways. It is possible that the midline RPn was also affected due to local diffusion of MN or imperfect centering of the cannulae about the midline. The weak attenuating effects of MN injections overall in the *Anterior* group suggests that nearby midline diencephalic structures (such as the superior colliculus or central gray) may also be involved in the expression of rigidity. Recent work by Ellenbroek et. al. [4] seems to support a role for GABAergic mediation of rigidity in this region. Injections at the 14 mm depth in the *Anterior* group fell very close to the median raphe nucleus (MnR) while those at the 16 mm depth in the *Posterior Extension* group were near the nucleus raphe magnus (RMg). Despite the absense of effect of MN injections at these sites, additional studies must be performed to accurately assess the role that these structures play in mediating opiate rigidity. Based on both the results of this study and previous work, it appears that hindbrain regions that contain serotonergic and GABAergic pathways mediate alfentanil-induced muscle rigidity in the rat.

1. Havemann U, Kuschinsky K. Neurochem Inter 4: 199, 1982.
2. Blasco TA, Lee D, Amalric M, Swerdlow NR, Smith NT, Koob GF. Brain Res 386: 280, 1986.
3. Weinger MB, Cline EJ, Smith NT, Koob GF. Anesthesiology 65: A344, 1986.
4. Ellenbrock B, Schwarz M, Sontag K, Cools A. Neurosci Letters 54: 189 1985.

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INJECTION SITE		AREA UNDER EMG CURVE	
		SALINE #	MN
ANTERIOR	12 mm	51.1 ± 8.6	23.6 ± 3.5 +
	13 mm	40.1 ± 5.9	25.7 ± 8.3
	14 mm	42.7 ± 6.6	34.4 ± 6.3
BILATERAL	12 mm	38.9 ± 2.6	28.7 ± 4.9
	13 mm	46.7 ± 6.6	21.6 ± 2.7 *
	14 mm	34.2 ± 5.6	23.5 ± 3.3 *
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.
\+ : $p < 0.05$ overall MN vs, SAL but no significant drug-site interaction
* : $p < 0.05$ between saline and MN groups at this site.