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Neuroanatomical Correlates of Morphine Dependence

Abstract. *Naloxone hydrochloride, an opioid antagonist, was applied to several discrete brain regions of morphine-dependent rats to precipitate abstinence. Severe withdrawal signs were elicited after administration in the thalamus but not in neocortical, hippocampal, hypothalamic, or tegmental areas of the brain.*

Physical dependence on morphine is manifested by a highly characteristic behavior when morphine intake is abruptly terminated or when a morphine antagonist is administered (*1*). The neuroanatomical areas related to the abstinence syndrome have not been clearly defined. Kerr and Pozuelo (*2*) reported that withdrawal signs, precipitated by opioid antagonists in the morphine-dependent rat, were suppressed or reduced when a major part of the ventromedial nucleus of the hypothalamus had been lesioned. Herz and his associates (*3*) postulated that structures in the caudal brainstem, most probably in the floor of the fourth ventricle, are important substrates for the development of dependence on morphine. In experiments in which crystalline naloxone hydrochloride was applied to discrete brain areas of the morphine-dependent rat, we find that the thalamus is one of the, if not the most sensitive, regions for precipitating withdrawal.

Male Sprague-Dawley rats (180 to 250 g) were used throughout these experiments. A 20-gauge stainless steel guide cannula, filed to a predetermined length, was stereotaxically implanted into the left hemisphere of the rat brain. Ether anesthesia was used for surgery. From 1 to 5 days after implantation of the guide cannula, dependence on morphine was induced by the subcutaneous implantation of a morphine pellet (*4*). To precipitate withdrawal, naloxone hydrochloride was applied to the brain 70 to 76 hours after pellet implantation. An inner cannula, 0.5 mm longer than the guide cannula, was tamped in crystalline naloxone hydrochloride and inserted into the guide cannula. This procedure was repeated twice to ensure that a sufficient amount of naloxone contacted the brain tissue. The total amount of naloxone hydrochloride inserted into the guide cannula ranged from 0.04 to 0.2 mg. While the precise amount of naloxone delivered to brain tissue cannot be ascertained, the treatment was uniformly administered to all animals, and the procedure successfully discriminated between brain areas of relative sensitivity. The methods and the problems associated with the application of chemicals to brain tissue have been discussed (*5*).

The abstinence syndrome precipitated by application of naloxone to the brain is similar to that observed after systemic administration of naloxone in morphine-dependent rats and will be described elsewhere (*6*). In brief, abstinence signs which are dose-dependent on naloxone, such as diarrhea, ear blanching, abnormal posturing, ptosis, teeth chattering, escape attempts, and wet shakes (*7*), appear within 10 minutes after cerebral application of naloxone in sensitive areas. Other abstinence signs such as seminal emissions and chromodacryorrhea may also be observed. Only salivation and licking movements during abstinence appear less intense when the cerebral, rather than the systemic, route of naloxone administration is utilized. Under identical experimental conditions, cerebral application of naloxone hydrochloride to the thalamus of six nondependent rats or repeated insertions of empty cannulas into morphine-dependent rats did not induce the abstinence syndrome.

The precipitated abstinence syndrome, as described above, was observed under standardized procedures. Rats were weighed and placed in 1-gallon mayonnaise jars, and naloxone hydrochloride was applied after a 10- to 15-minute adjustment period. Leaping attempts to escape from the jar and wet shakes are distinctive abstinence behavior and reflect a high degree of physical dependence. If a rat made two or more escape attempts or had three or more wet shakes within 10 minutes after cerebral application of naloxone it was considered to have undergone precipitated withdrawal and classified as exhibiting severe abstinence. This classification is based on experiments (*6*) which show that the median effective doses of naloxone for precipitating wet shakes and escape attempts are approximately five times greater than for other abstinence signs such as ear blanching, diarrhea, ptosis, swallowing movements, and teeth chattering. The anatomical correlates of

Table 1. Areas of precipitated abstinence in morphine-dependent rats.

Anatomical areas	Number in Fig. 1	Medial-lateral extension of area (mm)	Rats (No.)	Exhibiting severe abstinence (No.)
Neocortex	1	0–2.0	9	1
Hippocampus	2	0–3.0	12	0
Medial thalamus	3a	0–2.5	41	29
Lateral thalamus	3b	3.0–4.2	10	0
Diencephalic-mesencephalic junctures	4	0–2.0	20	13
Mesencephalon	5	0–2.5	16	0
Hypothalamus	6	0–2.0	7	0

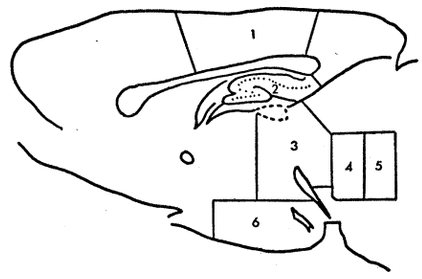

Fig. 1. Areas of precipitated abstinence in morphine-dependent rats. The figure represents a sagittal section of the rat brain 0.4 mm lateral to the midline and corresponds to figure 59b in the atlas prepared by Konig and Klippel (*8*).

these last-mentioned abstinence signs, which have lower thresholds for precipitation, were not investigated.

Animals were killed by decapitation, and the brains were removed and preserved in formalin. Cannula tracts were determined by gross dissection under a binocular microscope at 10.5 × magnification, and the site of the cannula tip was marked on diagrams depicting the frontal plane of the rat brain serially sectioned at 0.5 mm intervals. Representative tracts in some brain areas were embedded in paraffin, sectioned, and stained with cresyl echt violet for further examination. The stereotaxic atlas by Konig and Klippel (*8*) was used as the reference guide to anatomical localization.

The areas in the brain that were studied are illustrated in Fig. 1. The abstinence syndrome was most frequently evoked in and around the medial thalamic nuclei (area 3a and Table 1). The region surrounding the parafascicularis nucleus, in particular, was sensitive to naloxone precipitated withdrawal (*9*). Application of naloxone to lateral thalamic nuclei did not precipitate withdrawal (area 3b and Table 1), but positive responses were obtained in area 4 which is 1 to 1.5 mm caudal to the parafascicularis nucleus. Neocortical (area 1) and hippocampal (area 2) areas above the thalamus, as well as medial mesencephalic (area 5) and hypothalamic (area 6) regions, were not responsive to naloxone precipitated withdrawal.

Histologically, the insertion of the inner cannula into brain tissue produced a cylindrical lesion approximately 1 mm long and 0.5 mm wide in the tissue surrounding the cannula tip. Since the neocortical and hippocampal areas above the thalamus did not respond to naloxone, it is highly unlikely that the responses observed in the thalamus were due to diffusion of naloxone along the shank of the guide cannula. Most investigators (*10*) find that diffusion is limited when crystalline drugs are applied to brain tissue. The anatomical specificity of the behavioral responses to naloxone indicated that the responding elements were localized within the medial regions of the thalamus and rostral portions of the midbrain.

The precipitation of certain abstinence signs after regional application of naloxone to the brain indicated that the sites of adaptation to morphine have neuroanatomical specificity. It should be noted that only selected acute abstinence signs were systematically investigated. Dependence to other actions of morphine, as manifested by abstinence signs other than wet shakes and escape attempts, may develop in neural elements that are not exclusively located in the rostral brain. For example, the spinal cord may be involved in the persistence of some withdrawal signs described by Martin *et al.* as the chronic abstinence syndrome (*7, 11*). The abstinence syndrome precipitated by the cerebral application of naloxone, however, is almost identical to abstinence provoked by parenteral administration of naloxone. Although we chose to observe the abstinence signs which have the highest threshold for naloxone precipitation, other abstinence signs—such as teeth chattering, diarrhea, and ear blanching—appeared to increase, both in frequency and intensity, in the proximity of the sensitive thalamic structures.

Histochemical (*12, 13*) and physiological (*14*) studies of the medial thalamic nuclei provide a basis which indicates that these nuclei may be the site for some of the actions of morphine. The medial thalamic nuclei participate in the cortical recruiting response (*15*). Olds (*16*) and Stein (*17*) have shown that electrical or cholinergic stimulation of medial diencephalic structures have negative reinforcing properties on behavior. The parafascicularis nucleus in the medial thalamus receives cholinergic innervation from the tegmentum (*12*) and also has a relatively high cholinesterase activity (*13*). It has been known for some time that morphine inhibits the release of acetylcholine in nervous tissue and that opioid antagonists reverse this inhibition (*18*). If morphine should act by inhibiting the release of acetylcholine in medial thalamic nuclei, this might decrease the cortical reaction to inputs normally interpreted as noxious stimuli. It would not be unreasonable then to seek relationships between tolerance and physical dependence and the release of acetylcholine in medial thalamic nuclei.

EDDIE WEI
Division of Environmental Health Sciences, School of Public Health, University of California, Berkeley 94720
HORACE H. LOH
Mendocino State Hospital, Talmage, California
E. LEONG WAY
Department of Pharmacology, University of California Medical Center, San Francisco 94122

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