Antibodies to Cerebroside Sulfate Inhibit the Effects of Morphine and β-Endorphin

Abstract. *Morphine and β-endorphin inhibit the shaking response of pentobarbital-anesthetized rats to ice water. Stereotaxically guided administration of antibodies to cerebroside sulfate into the periaqueductal gray region, the most sensitive brain region in which to demonstrate inhibition of this response, antagonizes the effect of morphine and β-endorphin. These results suggest that cerebroside sulfate may be an integral component of an opiate receptor in rat brain.*

Although receptors of opiates and endorphin in the brain are generally acknowledged as proteinaceous in nature (*1*), there is compelling evidence that the acidic lipids phosphatidylserine and cerebroside sulfate (CS) also fulfill some of the accepted criteria for opiate receptors and may be an integral component of the true receptors (*2, 3*). Cerebroside sulfate meets most of the structural requirements proposed for an opiate-binding site (*4*). It exhibits a high affinity for and stereoselective binding to narcotic drugs; the binding is highly correlated with the rank order of analgesic potency for these drugs in man and rodent (*5*). A partially purified opiate receptor isolated from mouse brain (*6*) was identified as CS (*7*). A reduction in the availability of brain CS induced pharmacologically or genetically results in a decrease in the analgesic response to morphine (*8*). In this report, we present new, direct evidence based on an immunological approach that suggests a role for CS in the opiate and endorphin receptor mechanism.

Specific anatomical sites of opiate-opiate agonist action within the neuraxis have been the subject of many investigations. These studies usually involve observation of some pharmacological effect after direct application of morphine in specified areas of the brain. The periaqueductal gray (PAG) region of rat brain contains a high concentration of opiate and endorphin receptors, and is particularly sensitive to the inhibitory effects of opiates on the wet-shake response (quick rotational movement of the head or body) of pentobarbital-anesthetized rats to ice water (*9*). Since the PAG is accessible to stereotaxic microinjections, and since this effect of morphine is amenable to analysis, we used this brain region and the wet-shake response paradigm in an assay system to determine whether treatment with an antibody to CS would attenuate the response to morphine or β-endorphin.

An antiserum to CS was produced by repeated injections of a hapten composed of CS, phosphatidylcholine, cholesterol, and methylated bovine serum albumin (0.1:0.4:1.0:1.0 by weight). Antibodies to CS were purified by an immunoabsorption method and characterized. These antibodies fixed the complement and were active in agglutination tests (*10*). Male Sprague-Dawley rats (175 to 250 g) were used throughout the experiments; they were maintained in a 12-hour cycle of light and darkness and given free access to food and water.

The general procedure for observation of wet-shake behavior has been described (*9*). The rats were anesthetized with pentobarbital (48 mg/kg, intraperitoneally) and mounted in a David Kopf stereotaxic apparatus. The drugs and antibodies, dissolved in 0.85 percent saline, were administered directly into the PAG. A micrometer-driven syringe (Hamilton) attached to the stereotaxic device was calibrated by dilution with ^{14}C-labeled toluene. A hand-pulled siliconized glass cannula (tip diameter, 200 μm) was attached to the syringe. Different combinations of drugs, control solutions, and antibodies could be injected from the same cannula by introducing a small air bubble into the cannula between two solutions. All solutions were delivered slowly in a volume of 1 μl to prevent tissue distortion; the cannula was left in place for 1 minute after each injection to avoid refluxing up the can-

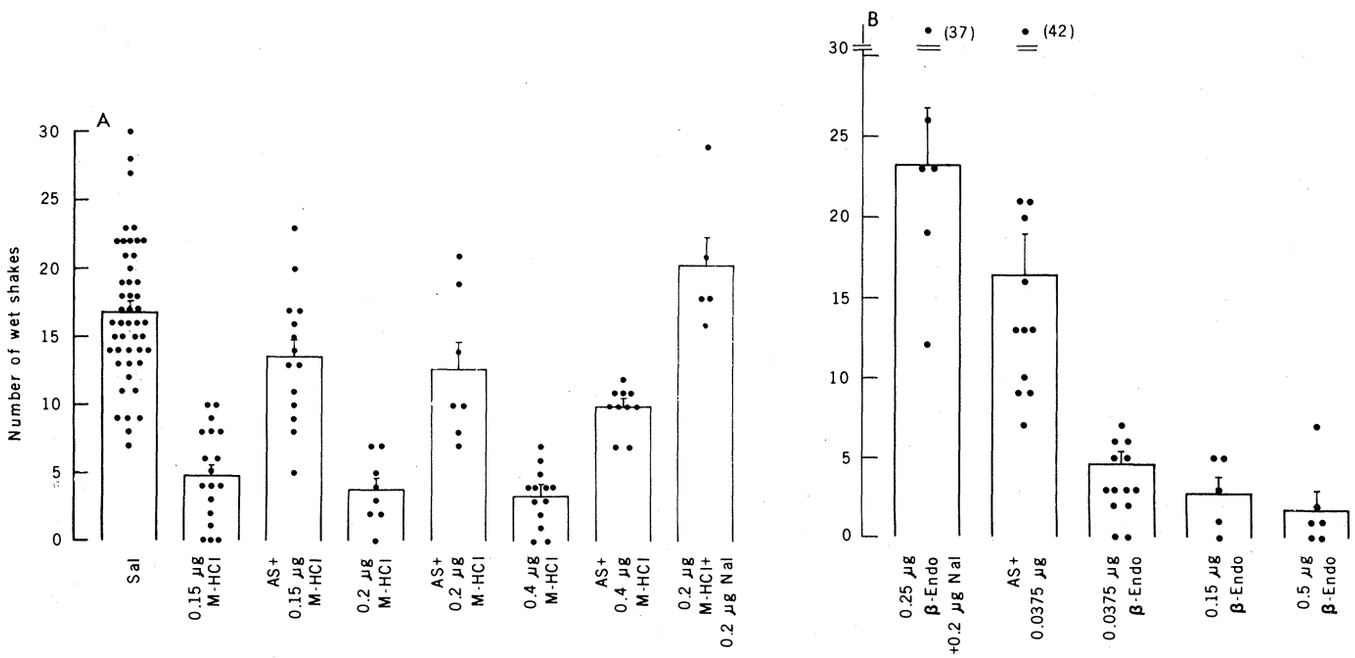

Fig. 1. Inhibitory effect of morphine hydrochloride (A) and β-endorphin (B) on wet-shake behavior in rats. Antagonism was by antibody to cerebroside sulfate (*AS*). A volume of 2 μl was delivered into the PAG in 1-μl increments with a 1-minute interval in between. The control consisted of saline (*Sal*). Morphine HCl (*M-HCl*) and β-endorphin were preceded by saline, AS, or naloxone (*Nal*) as indicated. The dose of morphine HCl and naloxone refers to the chloride salt. Each point is the datum for one animal. The AS + morphine HCl groups are all significantly different (*t*-test, $P < .005$) from the corresponding morphine HCl groups alone (A). The group receiving AS + 0.0375 μg of β-endorphin is also significantly different ($P < .005$) from the group receiving 0.0375 μg of β-endorphin (B).

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nula pathway. Two minutes after removal of the cannula, the rats, held by the nape of the neck, were immersed in an ice-water bath and an observer recorded the number of wet shakes occurring during a 5-minute period. The observer was not informed of the drug treatment. Cannula placement was verified in many of the rats after the experiment by gross histological examination of the PAG. In some cases, a dye was used to facilitate this examination.

As shown previously (*9*), morphine and human synthetic β-endorphin significantly inhibit the wet-shake response. The opiate antagonist naloxone, when administered prior to morphine or β-endorphin, completely prevents their effects (Fig. 1, A and B). One microliter of a solution of antibodies to CS (titer, 1:256 for CS in the complement-fixation assay) effectively antagonized the drug effect (Fig. 1, A and B). These antibodies do not react with morphine, naloxone, or β-endorphin in the heme-agglutination inhibition test. Our data indicate that it is the binding of the antibodies to some strategically located CS source within the PAG that prevents the normal interaction of morphine and β-endorphin with their respective receptors. The hypothesis that CS is the moiety which mediates the response to morphine or β-endorphin is further strengthened by the following observations: (i) an antibody to cerebroside, prepared similarly to the antibody to CS (*10*), was unable to antagonize the morphine inhibition of wet-shake behavior; (ii) denaturing the antibody to CS by heating it at 100°C for 20 minutes destroyed the ability of the antibody to antagonize morphine; (iii) an antiserum to another acidic lipid, triphosphoinositol (titer, 1:1200 for triphosphoinositol in the complement-fixation assay) (*11*), failed to antagonize morphine; and (iv) normal rabbit serum had no effect on the response to morphine (see Fig. 2A). Furthermore, the administration of naloxone or the antibodies to CS failed to elicit any response that was significantly different from that of the controls (Fig. 2B). This suggests that the effects of naloxone and antibodies to CS occur through an interaction with morphine and β-endorphin receptors.

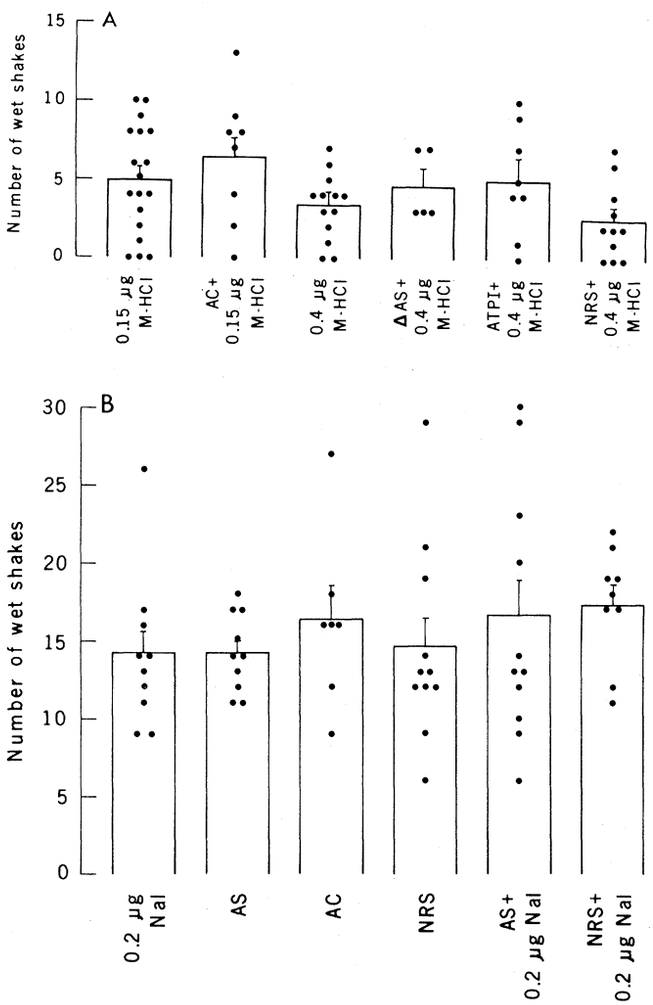

Fig. 2. (A) Failure of other immunoglobulins and boiled antibody to cerebroside sulfate (Δ*AS*) to antagonize response to morphine. Antibodies to cerebroside (*AC*), boiled AS, antiserum to triphosphoinositol (*ATPI*), and normal rabbit serum (*NRS*) were administered in 1-μl volumes to the PAG followed by morphine HCl (1 μg) 1 minute later. This treatment does not significantly alter the response to morphine. Each point is the datum for one animal. (B) Lack of effect of selected immunoglobulins and naloxone on the wet-shake response. Saline (1 μl) was injected into the PAG followed 1 minute later by 1 μl of AS, AC, or NRS. This treatment did not significantly alter the response. Combinations AS + naloxone and NRS + naloxone similarly were without effect. Each point is the datum for one animal.

The information contained in this report extends the previous observations (*2, 3*) on the putative role of CS in the opiate receptor by using an immunologically specific tool to alter a pharmacological response to morphine in vivo. The possible role of other lipids, such as phosphatidylserine, in certain opiate or endorphin receptors must also be considered (*2*). A preliminary report (*12*) has appeared in which it is shown that G_{M1} ganglioside may be involved in the analgesic response to morphine mediated by the PAG: it was discovered that a long-term attenuation of morphine analgesia occurred after injection into that region of antiserum to G_{M1} ganglioside. Thus, evidence that lipids may be an integral component of opiate receptors continues to accumulate.

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