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Antibodies to GMl Ganglioside Inhibit Morphine Analgesia

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KARPIAK, S. E. *Antibodies to GMl ganglioside inhibit morphine analgesia.* PHARMAC. BIOCHEM. BEHAV. **16**(4) 611–613, 1982.—It has been shown that the injection of antiganglioside serum into the periacqueductal gray matter of rats blocks morphine induced analgesia. This result is due to the action of antibodies to GMl ganglioside since the specific removal of these antibodies from the antiserum with pure GMl ganglioside eliminates the blocking activity. Specificity for GMl ganglioside was further shown by the blocking activity of choleragenoid, which binds specifically to GMl sites. Antibodies to other brain constituents, namely S-100 protein and myelin, did not block the morphine analgesia.

GMl ganglioside Antiganglioside Analgesia Morphine Morphine receptors

NUMEROUS studies have indicated that intracerebral injection of antibodies as interventive agents have the potential for selectively relating chemical structure to neurobiological process. Few reagents are available for which structural specificity of the antibodies is defined. One of these is antiserum to gangliosides where the specificity resides in the control, namely, antiserum from which anti-GMl ganglioside antibodies have been removed by absorption with pure GMl ganglioside. Such antibodies have been shown to induce seizure activity [5,7], to block the consolidation phase of memory on a passive avoidance paradigm [6], and to interfere with dendritic development in neonatal animals [8].

In this study we have examined the effect of injecting antiganglioside antibodies into the periacqueductal gray (PAG) matter of rats. The PAG region was selected as it has been well-established that small amounts of morphine injected into this region induce analgesia [4] and that the region has a high concentration of morphine receptors [12].

We have found that the injection of these antibodies into the PAG blocks morphine analgesia but does not, in itself, alter pain thresholds. Antibodies to other brain constituents, namely, S-100 protein and myelin did not block morphine analgesia.

METHOD

Animals

Male Sprague-Dawley rats (275–300 g) were used in all experiments.

Antisera

Antisera to total bovine brain gangliosides were prepared by the method of Pascal [11] as described in detail by Rapport [13]. The two antisera tested had titers greater than 350 (complement-fixation with 4 C'50 units of complement). The method of removal of antibodies from these antisera by absorption with pure GMl ganglioside has been described [7]. Antisera to S-100 protein and antiserum to myelin were the reagents described in a previous study [5].

Choleragenoid

Cholera toxin B-subunit was prepared from cholera toxin as described by Lai [9].

Behavioral Testing: Pain Threshold

Pain threshold was determined by the Flinch/Jump test [1,2]. Rats were tested in a Plexiglas box (7×7×8 in.) with a floor consisting of 10 grid bars through which scrambled electric foot shocks were delivered [14]. Using an ascending method of limits, only the jump response was used as a measurement criterion for these experiments. The jump response was defined as the lowest of two consecutive intensities (in mA) that elicited simultaneous withdrawal of both hind paws from the grid [1,2]. Each of ten trials began with a foot shock of 0.05 mA intensity and 500 msec duration, followed by increments of 0.05 mA every 10 sec. A trial was completed when two consecutive jump responses were recorded. The mean of the ten trials was taken as the jump threshold for that day. After three consecutive days of testing, the mean threshold on the 4th day (Day 4) was taken as the animal's pain threshold. All baselines were adjusted to a zero value for data analysis.

Injections and Test Schedule

After pain threshold baselines were determined, each rat was given (on Day 5) an injection of morphine sulfate (10 mg/kg, IP). One hour later the pain threshold was redetermined, and this value was taken as the base analgesic response to morphine. One week later, each rat was given an injection into the PAG of the reagent under test. For the injection into the PAG, rats were anesthetized with ether and

TABLE 1

Reagent	Analgesic Response to Morphine (mA) First Injection	Second Injection	Change in Analgesic Response (mA)	Significance
Antiganglioside No. 1855	0.35 ± 0.03*	0.11 ± 0.06	0.24	$p<0.01$
Antiganglioside Absorbed No. 1855	0.38 ± 0.08	0.35 ± 0.06	0.03	N.S.
Antiganglioside No. 1856	0.31 ± 0.05	0.08 ± 0.07	0.23	$p<0.01$
Antiganglioside Absorbed No. 1856	0.32 ± 0.05	0.29 ± 0.04	0.03	N.S.
Choleragenoid	0.38 ± 0.01	0.19 ± 0.06	0.19	$p<0.01$
Anti-S-100 No. 2045	0.35 ± 0.07	0.30 ± 0.04	0.05	N.S.
Anti-Myelin No. 1926	0.39 ± 0.07	0.37 ± 0.07	0.02	N.S.
Guinea Pig Serum	0.33 ± 0.05	0.34 ± 0.08	−0.01	N.S.
Saline	0.37 ± 0.08	0.39 ± 0.04	0.02	N.S.

*Mean analgesic threshold in mA S.E.M. N=five rats per group.

mounted in a Kopf small animal stereotaxic apparatus. PAG stereotaxic coordinates were taken in relation to bregma with the head in a horizontal position: posterior 6.5 mm; lateral 0.3 mm; vertical 5.8 mm. Test reagent was injected unilaterally (5 μl at 1 μl/min) using a 30 ga. injection cannula attached to a 10 μl Hamilton syringe. After the injection the cannula was left in place for 5 min to prevent fluid efflux.

Placement of the tip of the cannula was determined histologically. The data used were only from those animals where the location of the tip could be verified to have been within the limits of the lateral, dorsal or medial PAG.

Rats were retested for analgesic response to morphine sulfate (10 mg/kg IP) 10 to 14 days after the PAG injection. The difference in analgesic responses between the two injections of morphine was subjected to analysis.

RESULTS

Nine groups of rats containing 5 animals each were tested. Two of these groups were treated with two different antiganglioside sera, two with the absorbed antiganglioside sera (as controls), one each with antiserum to S-100 protein, antiserum to myelin, guinea pig serum (a rich source of complement activity), 1 μg of choleragenoid (a ligand with high affinity and specificity for GM1 ganglioside receptors), and one group with saline. In order to establish the stability of pain threshold baselines, all animals were tested 4 or 5 days after injection of reagents into the PAG. No significant deviation between the two determinations of pain threshold were observed, eliminating the possibility that either the reagents or the mechanics of injection had altered the PAG and thus were responsible for observed changes in response to morphine.

Rats which had received the native antisera (antiganglioside) and the choleragenoid showed reductions in analgesic response to the second injection of morphine of 0.19 to 0.24 mA ($p<0.01$; Table 1). In contrast, rats injected with absorbed antisera (from which antibodies to GM1 ganglioside had been removed), with native antiserum to S-100 protein, with antiserum to myelin, with guinea pig serum or with saline showed no significant change in their analgesic response (−0.01 to +0.05 mA).

Although the reduction caused by antisera to ganglioside was larger than that caused by choleragenoid (0.24 mA, 0.23 mA vs 0.19 mA) no statistical difference exists between the values.

DISCUSSION

The results of this study show that antiganglioside serum injected into the PAG has an inhibitory effect or blocking action on the analgesic effects of morphine but does not alter pain threshold. This result is due to the action of antibodies to GM1 ganglioside since the specific removal of these antibodies from the antiserum with pure GM1 ganglioside eliminates the blocking activity.

Specificity for GM1 ganglioside is further shown by the absence of blocking activity resulting from injection of

antisera against two other constituents of brain tissue, namely S-100 protein and myelin, and by the blocking activity of choleragenoid which has been shown to bind specifically to GM1 sites. The mechanism of action of antibodies to GM1 ganglioside in blocking the effect of morphine is not readily discernible. It could either be a direct action by combining with GM1 ganglioside molecules if they were elements of morphine receptors in the PAG, or indirect, through perturbation of membranes containing morphine receptors, in some way altering the conformation of these receptors to make them less effective. The most interesting feature of this study is that the action of the antibodies to GM1 ganglioside produced such a long-lasting effect since the retest of response to morphine was carried out 10 to 14 days after injection of antibodies into the PAG. The long duration of this effect is analogous to the long duration (several weeks) of the epileptiform discharges induced by antibodies to GM1 ganglioside injected into the sensorimotor cortex [5,7], suggesting that alterations in membranes induced by binding of antibody ligands are not readily reversible.

Previous studies have implicated other acidic lipids such as phosphatidyl serine and sulfatide (cerebroside sulfate) in opiate receptors on the basis of the effects of these acidic lipids on opiate binding and the action of degradative enzymes (especially phopholipiase A) on opiate binding [10]. The inhibiting effect on a wet-shake response to morphine of antibodies to sulfatide (injected into the PAG) has also been reported [3]. The duration of this inhibition was not studied.

The results suggest that although antibodies may be specific probes for the involvement of one or another type of molecule in receptor activity, the receptors themselves in their membrane orientation are sufficiently complex to resist such simple analysis. However, the discrimination that antibodies to GM1 ganglioside and to sulfatide display in their interventive action in various CNS functions, despite the lack of such discrimination in the distribution of molecules against which they are directed, reinforces the potential of immunological methods as an approach to functional dissection.

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