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Differences Between Two Substrains of AB Mice in the Opioid System

AXEL BECKER,* HELMUT SCHRÖDER,* MATHIAS BROSZ,† GISELA GRECKSCH* AND REGINE SCHNEIDER-STOCK‡

Institute of Pharmacology and Toxicology, †Institute of Biometrics and Medical Informatics, ‡Institute of Pathology, Otto-von-Guericke University, Leipziger Str. 44, Medical Faculty, 39120 Magdeburg, Germany

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BECKER, A., H. SCHRÖDER, M. BROSZ, G. GRECKSCH AND R. SCHNEIDER-STOCK. *Differences between two substrains of AB mice in the opioid system.* PHARMACOL BIOCHEM BEHAV **58**(3) 763–766, 1997.—Animals from two substrains of AB mice, i.e., ABH/Md and ABG/Md, differ in the occurrence of aggressive behavior. After maturation, male ABH mice regularly exhibited abnormal aggressive behavior making group-housing impossible. In contrast, ABG animals never showed such behavioral patterns. To elucidate the role of opioid mechanisms, we tested the reaction of these animals to morphine in the hot plate test. Moreover, specific DAMGO binding was measured. It was shown that mice from control groups differed significantly in reaction to the thermal stimulus. ABH mice had significantly longer reaction times. With increasing doses of morphine this difference disappeared, suggesting different levels of basal activity in endogenous opioid systems. This is underlined by significantly lower DAMGO binding in aggressive ABH mice. The results suggest that differences in endogenous opioid systems may account for differences in aggressiveness. © 1997 Elsevier Science Inc.

Mice Aggression Opioid system Analgesia

THE evidence on opiates and human aggression ranges from the earlier practice of using acute morphine as an antiaggressive drug to the high incidence of aggression and criminal behavior in narcotic addicts (9). Clinical observations have shown that a number of personality functioning shifts such as depersonalization disorders, antisocial personality disorders, passive-aggressive personality disorders, sadistic personality disorders, etc., predispose to psychoactive substance use (7,19). Furthermore, an association between childhood aggression and risk for subsequent development of a substance abuse disorder was found (11). Similarly, Young et al. (28) reported on a close relationship between aggression scores and substance abuse. This led to the assumption that aggression had some predicative influence on the incidence and severity of substance abuse (13), reflecting the link between aggressive behavior and opioid systems. Undoubtedly, it seems to be necessary to investigate the relation between opioid systems and the expression of aggressive behavior for a better understanding of the neurobiological basis of aggression.

To induce aggression in animals a number of models have been developed (9). However, most models are based on artificially induced aggression, making investigations on long-term effects of endogenous factors difficult. On the other hand, aggression towards other species (locust killing, mice killing) only reflects some aspects of aggressive behavior. Recently, we reported on aggression in two substrains of AB mice (1). After maturation, male ABH/Md (Hal) mice developed an abnormally high aggressiveness making group-housing impossible, whereas the closely related ABG/Md (Gat) substrain never exhibited aggression under such conditions (20). It appears that these AB mice might represent a useful experimental model for the study of aggression that is based on naturally occurring, endogenous factors. Moreover, closely related strains of mice offer a unique opportunity to investigate complex behavioral responses. ABH and ABG are highly similar, making it more likely that differences between these two strains will reflect a causal relationship.

In a locomotion test it was found that both substrains differed in reaction to dopaminergic and GABAergic stimulation, whereas in reaction to the unspecific CNS depressant hexobarbital no differences occurred (1). Because opioid mechanisms were shown to be involved in the mediation of

Requests for reprints should be addressed to Axel Becker, Otto-von-Guericke University, Medical Faculty, Institute of Pharmacology and Toxicology, Leipziger Str. 44, 39120 Magdeburg, Germany.

murine aggression (3,6,9,23), we tested animals from both substrains in their reaction to the antinociceptive property of morphine as measured in the hot-plate test. Other investigators reported that aggression was found to correlate with sensitivity to morphine analgesia (10). Moreover, to elucidate the activity of μ-opiate receptors the DAMGO binding in the hippocampus and the cortex was measured. It is well known that limbic structures but not the cortex are important for the modulation of emotional responses (e.g., as a result of thermal stimulation), which might involved in the occurrence of aggressive behavior.

METHOD

For all procedures followed, ethical approval was sought prior to the experiments according to the requirements of the National Act on the Use of Experimental Animals (Germany).

Subjects

These mice have been bred in our colony since 1984 and were kept under controlled laboratory conditions under a lighting regime of LD 12:12 (L 0600–1800 h), temperature 20 ± 2°C, and relative air humidity 55–65%. They had free access to commercial pellet food (Altromin 1326) and tap water. After weaning (21 ± 1 day) the animals were caged in groups of four to six males until they were 5 weeks old. Afterwards they were housed singly for 2 weeks. At the beginning of the experiments, the animals aged 7 weeks.

The protocol for selective breeding was described by Schneider et al. (20) and nomenclature of the strains is according to Schneider-Stock et al. (21).

Antinociceptive Testing

Antinociceptive reaction in mice was determined by the hot-plate test. The temperature of 56°C was controlled by a thermostat. All tests were performed between 0730–1100 h.

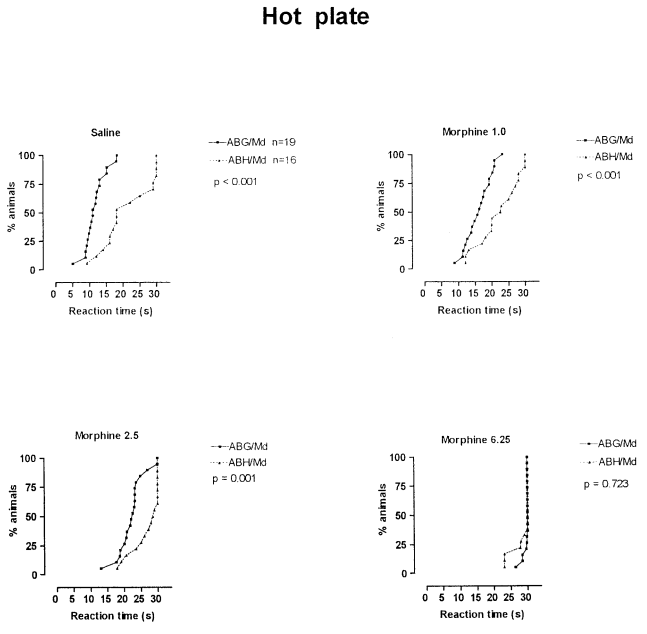

FIG. 1. Distribution function of animals with definite reaction times in the hot plate test. Percent of animals with definite reaction times, maximal reaction time was 30 s; doses in mg/kg.

Mice (ABG/Md = 19, ABH/Md = 16) were subcutaneously injected with morphine hydrochloride (1, 2.5, or 6.25 mg/kg, Synopharm Barsbüttel, Germany) solution or physiological saline for control. Injection volume was 1 ml/100 g body weight. The hot-plate procedure was performed 30 min postinjection and the end point used was the licking of the hindpaws or hindpaw trembling. The animals were taken off immediately after the first sign of reaction or, if there was no reaction, after 30 s.

Because of the use of the same animals with different doses of morphine the mice were injected in a randomised schedule. The period between each test was 1 week.

(^{3}H)-DAMGO Binding Assay

Another group of mice (n = 8 per group) was decapitated, their brains were rapidly removed, and the hippocampi and cortices (frontal, acoustic, visual) were dissected out. To prepare a 3% homogenate (wet w/v), tissue was homogenised in 50 mM Tris-HCl buffer, pH 7.8, containing 2 mM EDTA, 1 mM EGTA and 5 mM $MgCl_2$ (Sigma Chemie, Steinheim, Germany) and stored at −70°C. After thawing, the homogenates were centrifuged for 20 min at 50,000 × g. The resulting pellet was washed four times with homogenization buffer and centrifuged again.

The (^{3}H)-DAMGO (Tyr-D-Ala-Gly (Me)Phe-Gly-ol) binding was measured using a modified method described by Simantov et al. (24). The radiochemical purity of the ligand was checked by HPLC and was found to be about 91%.

The pellet was suspended with Tris-HCl buffer and 50 μl-aliquots of the crude membrane suspension containing 150–250 μg protein were then mixed with (^{3}H)-DAMGO (specific activity: 1.43 TBq/mmol, NEN-Dupont, Bad Homburg, Germany) and were subsequently incubated for 40 min at 25°C. All assays were performed in at least duplicate. Specific binding was calculated by subtracting nonspecific binding—defined as that seen in the presence of 2.5 nM (^{3}H)-DAMGO plus 1 μM unlabeled DAMGO—from total binding obtained with (^{3}H)-DAMGO alone.

The reaction was terminated by rapid filtration under reduced pressure through 0.1% PEI treated GF 10 glass-fiber filters using an Inotech harvester (Berthold, Wildbad, Germany). The filters were washed with buffer and taken for liquid scintil-

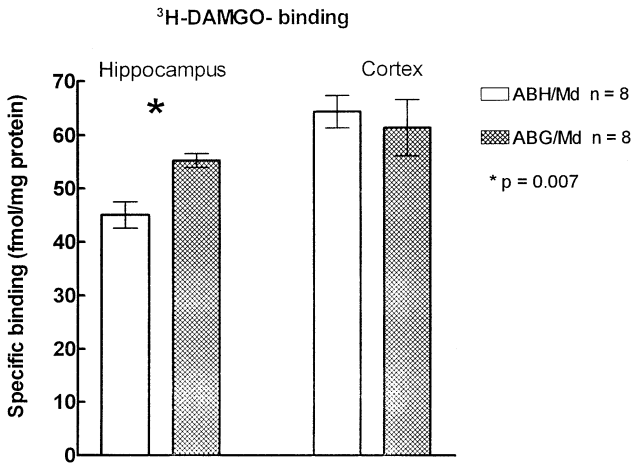

FIG. 2. ^{3}H-DAMGO binding in different brain structures of ABH/Md and ABG/Md mice.

lation counting in a solvent containing toluene. The data was determined as fmol bound radioligand per mg protein.

Statistics

To evaluate hot plate data, the Log rank test (due to of censored data, maximal reaction time was 30 s) was performed. Strain differences in DAMGO binding were analyzed by the Mann–Whitney *U*-test. The significance level was fixed at 0.05.

RESULTS

In control groups, significant differences between Hal and Gat were found (log rank = 19, 62, $df = 1$, $p < 0.0001$) in reaction to a thermal stimulus. Gat mice had shorter reaction times (Fig. 1). Equivalent results were obtained after having injected 1 mg/kg morphine (log rank = 12,78, $df = 1$, $p = 0.0004$) and 2.5 mg/kg morphine, respectively (log rank 10.04, $df = 1$, $p = 0.0015$). Considering the curves in Fig. 1, it becomes obvious that differences between animals of both substrains declined with increasing morphine test doses. After injection of 6.25 mg/kg morphine most animals reached the end point of testing, i.e., 30 s, indicating strong analgesic effects. At this point, there is no difference between both substrains (log rank = 0.13, $df = 1$, $p = 0.7227$).

As shown in Fig. 2, specific (^{3}H)-DAMGO binding in the hippocampus formation is significantly lower in Hal ($U = 9$, $p = 0.007$). Considering DAMGO binding in the cortex, no difference was found ($U = 18$, $p > 0.05$).

DISCUSSION

Aggression is considered a complex behavioral response to environmental stimuli. It is aimed at satisfaction of different individual needs such as territorial needs, competition for mates, competition for food, defence, etc. It was suggested that different categories of aggressive behavior might be based on different central neuronal mechanisms and neuroendocrine modulation (12).

A number of research strategies have been employed to shed light on the role of specific neurotransmitters in aggression. It was found that serotonergic, GABAergic, dopaminergic, cholinergic, and noradrenergic mechanisms are involved in mediation and regulation of this complex behavioral pattern (2,8,9,14–16,18,22,25–27).

Specifically, Hal and Gat mice differ in response to diazepam and haloperidol (1). This might explain differences in behavior because the GABA/benzodiazepinergic and the dopaminergic neurotransmission system are strongly involved in aggression. However, it remains unclear whether both systems act per se.

Beside GABA, opioid systems are differently involved in the mediation of various components of aggression. It was suggested that δ receptors have a possible physiological role in affective and defensive reactions (5). Considering maternal aggression, it was suppressed by morphine demonstrating receptor involvement in this type of postpartum behavior (4). Moreover, Miner et al. (10) concluded that the sensitivity to morphine-induced analgesia appear to be related to the degree of aggression to which mice were exposed.

As mentioned above, male Hal mice exhibited after maturation excessive aggression making group-housing impossible. For that reason, following the fifth weeks of age the animals of both substrains were housed singly. From the literature it is known that single housing altered functioning of the endogenous opioid system (17). As shown in Fig. 1, significant differences in reaction to the thermal stimulus were found in control groups, indicating different functioning of the protective system in these mice. Parallel to nociceptive reactions, DAMGO binding is significantly higher in nonaggressive Gat mice (Fig. 2). The differences found in our experiments are independent of housing conditions because animals from both substrains were sheltered under identical conditions.

Isolation represents a frequently applied method for the induction of aggressive behavior. There is evidence that isolation produced a decrease of GABA, the main inhibitory neurotransmitter, in different brain structures of mice (25). In an earlier study (1), we showed significant differences between Hal and Gat mice in response to diazepam. Thus, it seems likely that differences in the level of central inhibition (GABAergic and opioid systems) might be, in part, responsible for differences in aggressiveness in AB mice.

As shown in Fig. 1, aggressive Hal mice needed significantly longer times to reaction to the thermal stimulus. However, after injection of morphine the difference between the animals became smaller and in animals dosed with 6.25 mg/kg morphine a considerable analgesic effect was found. This suggested different level of basal activity of the endogenous opioid system rather than ineffective functioning of opioid systems. Alternatively, a reduction in the efficacy of pain transmitter systems (e.g., serotonine, substance P) might contribute to the differences in basal response. Thus, subsequent investgation will aim at investigating the differences in basal response concerning opioid sensitiveness.

Taken together, mice from the Hal and Gat substrain seem to be useful for the study of basic mechanisms involved in expression and modulation of aggressive behavior with special reference to the endogenous opioid system. Moreover, congenic AB mice, which are not aggressive, were bred (21), and these mice might be an excellent model to study the genetic background of aggression and its neurobiological basis.

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