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The genetics of pain and pain inhibition

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ABSTRACT **The present review summarizes the current state of knowledge about the genetics of pain-related phenomena and illustrates the scope and power of genetic approaches to the study of pain. We focus on work performed in our laboratories in Jastrzebiec, Poland; Portland, OR; and Los Angeles, which we feel demonstrates the continuing usefulness of classical genetic approaches, especially when used in combination with newly available molecular genetic techniques.**

It is widely appreciated among clinicians and researchers that sensitivity to pain and responses to analgesics are highly variable. Beecher (1) reported that some soldiers wounded in battle did not require morphine, and Lasagna and Beecher (2) found that standard 10-mg doses of morphine inadequately relieved postoperative pain in up to 35% of patients. Conversely, some people exhibit marked analgesia from placebo alone (3). The analgesic efficacy of morphine and other opiates is known to depend on many factors related to the drug, the patient, and the nature of the pain itself (4, 5). With respect to patient-related variability, some combination of genetic factors—e.g., sensitivity to the noxious stimulus, inborn personality variables, and gender—and environmental influences—e.g., learned personality variables, gender and attractiveness of the experimenter, subject's mood during testing, and time of day—must be responsible. A similar constellation of genetic and environmental factors no doubt underlies the considerable variation noted in clinically significant pain syndromes. Studies show significant correlations between pain report and behavior of family members (6, 7), but interpretations of shared socialization have tended to predominate over genetic explanations.

In fact, the study of genetic differences in pain-related traits has been largely neglected. One reason may be that pain research has been blessed by the discovery of endogenous opioid peptides and the development of an abundance of increasingly specific opiate receptor ligands. Genetic approaches to the study of pain, therefore, have been largely overlooked in favor of pharmacological interventions. In general, these experiments have been quite successful, elucidating the physiology and neurochemistry of pain transmission and modulation to an impressive degree of detail (8, 9). There exist, however, some aspects of pain neurobiology that cannot be adequately addressed without the use of genetic techniques. Standard biological techniques attempt to obtain information about all members of the species under study; in effect, to learn about the "universal rat." The very purpose of the genetic approach, on the other hand, is to explain individual differences. As such, genetic techniques are a perfect complement to physiological and pharmacological investigations.††

The Scope of Genetic Approaches

The first and most obvious use of genetic approaches is to establish whether a trait of interest is determined by nature or nurture. As is now commonly appreciated, virtually all traits are influenced by both genetic and environmental factors. Classical genetic techniques in humans and animals can, however, be used to determine the heritability of a trait, defined as the percentage of the total variance attributable to genetic factors (10). A number of pain-related traits are known to have comparatively high heritability and, thus, are open to genetic investigation. Once a trait has been shown to be at least partially determined by genetic factors, biometric analyses can be performed to determine the mode of inheritance—i.e., dominant or recessive—effects of natural selection, and how many genes mediate the trait (11). Another use of genetic techniques is to establish correlation or dissociation between traits; the genetic covariation of two traits represents evidence of a functional relationship between them (12, 13). Such studies have proven to be especially useful for the generation of hypotheses regarding underlying physiological mechanisms. Finally, the use of classical genetic techniques can render a trait amenable to powerful molecular genetic analyses. It is now possible to map trait-relevant loci onto the genome of many organisms (especially mice and humans), even for traits determined by multiple genes and even in the absence of plausible candidate genes (14, 15). Once mapping is accomplished, the genes controlling the trait of interest can be identified and cloned, and the specific influence of each gene on the trait can then be examined in isolation. Thus, employing classical and then molecular genetic techniques, we should be able to achieve a fuller understanding of variability in pain-related phenomena at all levels of biological analysis: from DNA sequence variations (genotype) to altered behavior (phenotype).

Genetic Models

To study individual differences among members of a population, genetic models must be available. Genetic models can be

Abbreviations: B6, C57BL/6; D2, DBA/2; HA/LA, high analgesia/low analgesia; HAR/LAR, high analgesic response/low analgesic response; RI, recombinant inbred; SIA, stress-induced analgesia; PAG, periaqueductal gray matter; QTL, quantitative trait locus; SPA, stimulation-produced analgesia; POMC, proopiomelanocortin; DAMGO, [D-Ala^2, N-Me-Phe^4-Gly-ol]enkephalin; DADLE, [D-Ala^2, D-Leu]enkephalin; DPDPE, [D-Pen^2, D-Pen^5]enkephalin.

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††This inaugural article presents an overview of research on genetic aspects of pain and pain inhibition that was begun in collaboration with B.S. in Jastrzebiec, Poland, continued in the J.C.L. laboratory at UCLA for several years, and is now being pursued by the first and principal author of this paper (J.S.M.), currently in the J.K.B. laboratory in Portland, OR.

either identified or produced in laboratory animals, but in humans, of course, models can only be identified. Once "affected" individuals are discovered, standard human behavioral genetic techniques can be employed—e.g., pedigree analysis, linkage studies, and twin/adoption studies. A number of case reports of familial neuropathies exist (see refs. 16 and 17), but in these the inherited trait results in a neuropathology that may be painful but is not likely to be relevant to normal variation in pain sensitivity. One fascinating but rare inherited syndrome with possible relevance is congenital insensitivity to pain, defined by Thrush (18) as the absence of pain sensation from birth unaccompanied by loss of other sensory modalities or demonstrable peripheral nerve pathology. In an intriguing placebo-controlled, double-blind study, a patient with spontaneous elevation in the nociceptive flexion reflex of 350% compared with controls exhibited significant decreases in reflex thresholds in response to the opiate antagonist naloxone (19). The investigators suggested that the lack of pain perception in this patient was due to a tonic hyperactivity of endogenous opioid analgesic mechanisms. Unfortunately, naloxone has been without effect in other such patients (see, for example, refs. 20 and 21). One genetic polymorphism with possible relevance to opiate sensitivity has been well studied in humans. About 10% of Caucasians are poor metabolizers of the liver isozyme P450IID6 (sparteine/debrisoquine oxygenase), due to a mutation in the *CYP2D6* gene (22). The O-demethylation of the widely used opiate drug codeine to morphine requires this enzyme (23). Poor P450IID6 metabolizers are largely unable to bioactivate codeine, and for these people codeine is an inefficient analgesic (24). In addition, poor metabolizers have been shown to be less tolerant to tonic cold pressor pain, possibly due to defective endogenous synthesis of morphine (25), which has been identified in several animal species, including man (for example, ref. 26). In general, however, although large individual variation is commonplace in laboratory studies of human pain and analgesic sensitivity (27), the sources of this variability have not been subjected to systematic investigation.

In contrast to the situation in humans, a number of genetic models of pain-relevant traits have been identified or produced in animals. The typical subject for mammalian genetics research is the common house mouse, *Mus musculus* (see ref. 28). This species is favored for genetic studies because of its small size and carefully catalogued pedigrees. It is important to note that the laboratory mouse differs from the ubiquitous laboratory rat in many ways, including some pain-relevant traits. Such species differences themselves represent a genetic model, albeit one of limited usefulness. More useful intraspecific genetic models that have been employed to study pain include the following:

Inbred-strain comparisons. Inbred strains are created by repeated sibling matings for at least 20 generations. The resultant offspring from a given strain are virtually genetically identical to each other. The specific allele—i.e., gene—that becomes fixed at each genetic locus is randomly determined; therefore, individuals from different inbred strains will likely differ at many loci. The comparison of inbred strains is thus tremendously useful in addressing nature-versus-nurture issues. Differences among members of the same inbred strain must have an environmental origin—e.g., measurement errors, home cage microenvironment, birth order, cage dominance—differences between inbred strains are likely due to genetic factors alone when strains are raised and tested under the same conditions. The first step in the genetic investigation of a trait is often a comparison of responses of a panel of inbred strains; a genetic similarity matrix can be consulted to identify strains which will likely differ (29).

Recombinant inbred (RI) strains. The existence of large and reliable inbred strain differences has encouraged the production of more complex but more useful genetic models, such as RI strain sets (30, 31). Each strain in an RI set represents the inbred descendants of an F_2 intercross between two inbred progenitor strains. In effect, each strain of an RI set represents a unique genetic rearrangement of the progenitor alleles frozen in time. As such, linkage analysis can be performed with this model without the need to make new test crosses. RI sets, especially the 11-strain CXB series [derived from a BALB/c × C57BL/6 (B6) cross] and the 30-strain BXD series [derived from a B6 × DBA/2 (D2) cross], have been profitably used to identify major gene effects and quantitative trait loci (QTLs, loci contributing small influences on a continuously distributed trait) mediating pain-related traits (see below).

Selective breeding. Also known as artificial selection, selective breeding is the most direct way to study the genetic determinants of a trait (10, 32). In this approach, one breeds only those individuals displaying extreme responses of a trait of interest. Selection usually proceeds from a genetically heterogeneous founder population bidirectionally; that is, in both high and low directions concurrently in two oppositely selected lines. By selective breeding, one can specifically alter the frequency of only those genes directly influencing a trait of interest, instead of merely examining (or in the case of RI strains, producing and then examining) serendipitous genetic rearrangements affecting that trait. It is important to note that genes are not being created, destroyed, or altered; the only effect of selection is to change trait-relevant gene frequencies in an experimental population. If selection is successful, genetic control over a phenotype is demonstrated *prima facie*, and the speed of selection progress—i.e., the rate of divergence in the high and low lines—provides a rough estimate of the number of genes responsible for the magnitude of the phenotypic response. Selective breeding, like other genetic models but in a direct-versus-indirect manner, also allows for the examination of the pleiotropic effects of genes. That is, if traits other than the selected trait also show divergence, it is likely that these "correlated traits" share similar genetic determinants with the selected trait (12, 13).

Spontaneous mutants. A number of point mutants of the B6 strain—i.e., mutations on a B6 genetic background—have been identified by alert animal technicians, and are maintained by The Jackson Laboratory (33). Surveys of such mutants have identified some that display differential opiate analgesic sensitivity. These include the mutations Sepia and Gunmetal (34), Jimpy (B6CBA-$A^{W\text{-}J}$/A-Ta jp) (35), and Beige-J (C57BL/6J-bg^{J}) (36). Sepia and Gunmetal mice, bearing mutations on chromosomes 1 and 14, respectively, display more potent morphine analgesia than do B6 mice (34). Beige-J mice display markedly less opiate analgesia than do B6 mice (36, 37), a deficit that seems to be related to an abnormal circulating splenic factor (38).

Targeted mutations. One needs to be quite fortunate to find a known mutant displaying altered responses on a particular trait. Although it is possible to mutagenize animals and screen for altered responses, this approach is quite costly and time-consuming. If, however, a gene relevant to a trait of interest has been cloned, a powerful new method exists to investigate the role of that gene directly. It is now commonplace, if not exactly simple, to produce transgenic mice containing exogenous genetic material added randomly to the genome or "knockout" mice containing null alleles (39). The resultant targeted mutants may show gains or losses of function, respectively, confirming the role of the targeted gene in the trait. In addition, the identification of compensatory responses to the overexpression or lack of expression of a gene can be quite informative. In combination with antisense approaches (see, for example, ref. 40), this technology promises to be very useful for the genetic dissection of pain-related traits.

Other models. A number of other genetic models have been described, and some have been used to investigate pain-related phenotypes. For instance, mice of a subline of the B6 inbred

strain maintained separately by Bailey (C57BL/6By) for over 40 years may display higher analgesic sensitivity to morphine than their counterparts at The Jackson Laboratory (C57BL/6J) (41). Congenic lines, in which a small chromosomal region from one strain is transferred onto a background strain by repeated backcrossing (42), are being developed in our laboratory (J.K.B.) by using regions showing linkage to morphine analgesic magnitude on chromosomes 9 and 10. Finally, a few studies describe so-called "vendor effects," in which rodents of the same outbred strain obtained from different suppliers show differences in pain-related responses (43–45).

CXBK Mice. Our laboratories, like many others, were first attracted to opiate pharmacogenetics by the startling deficiencies in morphine responses exhibited by the CXBK mouse, one strain from the CXB RI set. Developed by Bailey (30) in the early 1970s to map histocompatibility genes, the CXB set contains, in addition to the BALB/c and B6 progenitor strains, seven RI strains and two reciprocal (BALB/c × B6)F_1 hybrids. In the first demonstration of genetic differences in an opiate phenotype, Bailey's laboratory (46) determined that the 11 strains of the CXB series fell into three statistically significant groupings with respect to whole-brain homogenate [^{3}H]naloxone binding (B_{max}): CXBH > all others > CXBK. In addition to having the lowest opiate binding, CXBK mice displayed the lowest analgesic response to morphine as measured by the tail-flick assay.

The deficient opiate binding in CXBK mice was soon replicated and extended by a number of groups. Brain membrane preparations from CXBK mice poorly bind tritiated [D-Ala^2,N-Me-Phe^4,Gly-ol]enkephalin (DAMGO) (47), dihydromorphine (48), ethylketocyclazocine (48, 49), and [D-Ala^2,D-Leu^5]enkephalin (DADLE) (48). Autoradiographic analysis has shown that compared with B6 mice, CXBK mice display equal or decreased dihydromorphine binding in every brain area studied (50). Loci of possible relevance to pain processing showing pronounced CXBK binding deficiencies include the ventral periaqueductal gray matter (PAG); raphe nuclei; substantia gelatinosa; and laminae I, II, and V of the spinal cord. These authors (50) further conclude that the opiate binding deficits exhibited by CXBK mice seem to be specific for μ_1 sites relative to μ_2 or δ sites. Concurrent with this hypothesis, CXBK mice show poor analgesic sensitivity to intracerebroventricular injections of the highly μ-selective agonist DAMGO but unaltered sensitivity to the δ_1-selective agonists, [D-Pen^2,L-Pen^5]enkephalin (DPLPE) and [D-Pen^2,D-Pen^5]enkephalin (DPDPE) (51, 52). However, these mice may have a δ_2 receptor deficit, as indexed by the lack of analgesic efficacy of [D-Ala^2,Glu^4]deltorphin (52). Additionally, CXBK mice may have κ-receptor deficits since they show hyporesponsivity to the effects of the κ-selective agonist U-50,488H (53, 54) (but see ref. 37), and the κ_3-selective agonist naloxone benzoylhydrazone (54). CXBK mice do appear to have normal levels of endogenous opioid peptides (55). The genetic μ receptor deficiency in CXBK mice appears to be supraspinal, as intrathecally administered morphine is equianalgesic in CXBK, BALB/c, B6, and CD-1 mice (51, 56).

We embarked on a number of studies using this intriguing model. Compared with B6, BALB/c, or CXBH mice, we found CXBK mice to display deficient or absent morphine analgesia (lower sensitivity by a factor of 42) (57), morphine hyperlocomotion (57) (see also ref. 58), opioid footshock stress-induced analgesia (SIA) (ref. 59; see also ref. 60), opioid swim SIA (61, 62), and ethanol-induced analgesia and hypothermia (63). An interesting secondary finding of many of these investigations is that despite lacking functional opioid analgesic mechanisms, CXBK mice nonetheless do exhibit naloxone-insensitive, nonopioid analgesia. In one study, for instance (64), the threshold current required to elicit stimulation-produced analgesia (SPA) from the PAG did not differ among CXBK, B6, BALB/c and CXBH strains unless mice were pretreated with naloxone. Following blockade of opiate receptors, all strains but CXBK showed large increases in SPA current threshold, suggesting that these other strains were employing opioid SPA mechanisms, whereas the SPA in CXBK mice was wholly nonopioid. These data support and extend previous findings of the existence and interaction of multiple pain-processing systems (9). Just as the chronic blockade of opiate receptors can potentiate nonopioid swim SIA, a phenomenon termed collateral inhibition (65), the absence of opioid analgesia via genetic endowment can apparently result in the substitution and/or upregulation of alternative nonopioid mechanisms.

Other findings of deficient or absent opiate responses in CXBK mice include the following: acupuncture analgesia (66), enkephalinase analgesia (67), conspecific defeat SIA (68), immunomodulation (69), saccharin intake (70), and some measures of opiate withdrawal (jumping, body shakes) (71, 72). CXBK mice appear not to differ from their progenitor strains with respect to basal nociceptive sensitivity (67, 72), nitrous oxide analgesia (53), morphine-induced respiratory depression (57), morphine-induced hypothermia (J.K.B., unpublished data), morphine's antitussive effects (73), or the reinforcing effects of morphine as reflected in operant and conditioned place preference studies (refs. 74 and 75, but see ref. 76). Such negative findings are usually interpreted as evidence that the phenomena in question are not mediated by μ_1 receptor mechanisms. Indeed, CXBK mice have been used quite profitably to support the involvement of opiate (especially μ_1) receptors in a behavior or physiological response in which these animals prove deficient (see also refs. 77–79) and to imply the participation of μ_2 receptors in the mediation of responses in which CXBK mice appear normal (see also refs. 80 and 81). In effect, CXBK mice have been used for 20 years in much the same way as transgenic knockout mice are now.

There are some important limitations to conclusions obtained from experiments using CXBK mice, however. First, as noted by Baran *et al.* (46) in the seminal investigation, the correlation in the 11 CXB RI strains between [^{3}H]naloxone binding and morphine analgesic magnitude was positive (r = 0.48) but not statistically significant. The lack of significance could simply reflect insufficient statistical power; indeed, this correlation is significant in the 30-strain BXD RI panel (82). Ongoing experiments in the J.K.B. laboratory, however, cast doubt on the generally accepted causal relationship between the paucity of μ receptors and deficient analgesic sensitivity in CXBK mice. Test crosses between CXBK × B6 mice segregate in expected Mendelian fashion for a single-locus trait, supporting the previous contention that the CXBK strain is an outlier due to the effects of a new mutation arising during the development of the CXB RI series (83). However, we have found that whole-brain naloxone binding does not cosegregate with morphine analgesia in backcross animals (J.S.M. and J.K.B., unpublished results). It is possible, therefore, that CXBK mice exhibit deficient opiate analgesia for reasons not directly related to their poor opiate binding. Alternatively, it may be that these two traits are both caused by a third, unknown factor. We are currently mapping the morphine analgesia phenotype in segregating crosses to address this issue.

High Analgesia/Low Analgesia (HA/LA) Mice. Animals exposed to a variety of environmental stressors display decreased sensitivity to pain, and it is hypothesized that endogenous pain inhibitory circuitry in the central nervous system (CNS) exists to mediate this phenomenon (see ref. 84). A number of stressful manipulations have been employed in the laboratory to study SIA, and experiments reveal large individual differences in SIA magnitude. Thus, to directly investigate the inheritance of endogenous pain inhibition, we selectively bred for high and low expression of SIA. Starting with a heterogeneous, outbred population of 150 Swiss–

Webster mice, we bred for high analgesia (HA) and low analgesia (LA) (measured on the hot-plate test) resulting from a 3-min forced swim in 20°C water (85). In each generation of selective breeding (by mass selection), mice with postswim latencies ≤10 s and ≥50 s were mated to form or continue the LA and HA lines, respectively. To minimize inbreeding, 10 breeding pairs per line were used in each generation. To control for environmental effects, an unselected—i.e., randomly bred—control (C) line was maintained concurrently. Significant divergence in SIA magnitude between HA and LA mice was achieved with only one generation of selection (85). This fact argues strongly both for the high heritability of this trait and for the possibility that this trait is mediated to a large extent by relatively few gene loci (10, 32). The involvement of other minor genes in SIA is revealed by the increasing divergence with successive generations of selection (85); indeed, the HA line may not have reached asymptotic responding until the 21st selected generation (B.S., unpublished data). The HA selection appeared to progress faster than that of the LA selection (relative to unchanging C levels). This asymmetrical selection progress may imply that natural selection favors low magnitudes of SIA over high (32). However, wild field mice (*Apodemus agrarius*) and wild house mice exhibit HA-like levels of swim SIA (86), arguing that selection in a laboratory setting may not be adequately representative.

The large differences between HA and LA mice in swim SIA magnitude on the hot-plate and tail-flick tests (85)—virtually all HA mice now display maximal latencies after swim stress, whereas swim SIA in LA mice is now undetectable—have established these lines as an important genetic model of endogenous pain inhibition. A number of experiments were performed to establish genetic correlations between swim SIA and other presumably similar traits. In studies looking for correlated responses, a major theoretical limitation concerns the possibility that genes being fixed by genetic drift rather than genes being fixed by the selection itself are affecting the correlated trait (12, 32). The preferred method to control for this possibility is the maintenance of replicate selection lines. These were unavailable for the HA/LA project due to space limitations, but Henderson (13) has argued that correlated responses with magnitudes of divergence approaching that of the selected trait are in fact trustworthy. Using this criterion, we have identified a number of traits that appear to be mediated by genetic mechanisms similar to those mediating swim SIA, including baseline nociceptive sensitivity to thermal stimuli (refs. 85 and 87; but see ref. 88), morphine analgesia (87, 89–91), levorphanol analgesia (92), DAMGO analgesia (93), U-50,488H analgesia (94), opioid footshock SIA (95, 96), opioid SPA from the PAG (97), opioid swim SIA tolerance (98) (but not morphine tolerance; ref. 91), and ethanol-induced analgesia (99). The differential morphine analgesia displayed by HA and LA mice is particularly noteworthy: analgesic potency ratios—i.e., LA ED_{50} to HA ED_{50}—for systemic morphine on the hot-plate test are estimated as being variously 8 (87) and 35 (B.S., unpublished data). As in CXBK mice, the genetic alterations affecting morphine analgesia in HA/LA mice appear to be of supraspinal origin, since intrathecally administered morphine is isoanalgesic in these lines (B.S., unpublished data).

Conclusions from experiments using the HA/LA lines are hampered by the complicated nature of the trait under selection. On the basis of evidence available at that time (100), the 3-min duration and 20°C water-temperature parameters were chosen to produce opioid-mediated SIA. It has become clear, however, that in our hands these swim-stress parameters produce a mixed opioid/nonopioid phenomenon. That is, SIA resulting from 3-min swims in 20°C water is partially reversed by the opiate antagonists naloxone and naltrexone and partially insensitive to such antagonism (but blocked in male mice by the *N*-methyl-D-aspartate antagonist MK-801) (89, 90, 101). Therefore, the frequencies of genes mediating nonopioid forms of SIA are likely also being altered by this selection project. We have demonstrated that opioid and nonopioid swim SIA can be differentially elicited by altering the severity of the swim stress. Mildly stressful parameters (short swim durations and/or warm water temperatures) specifically produce opioid SIA, whereas more severe parameters (long swim durations and/or cold water temperatures) specifically produce nonopioid SIA (101, 102). By using these protocols, we have shown that HA/LA mice show equally divergent opioid and nonopioid SIA (101).

With this caveat in mind, many experiments have been undertaken to identify the genetic mechanisms underlying the differential phenotypes exhibited by HA and LA mice. One obvious question is whether these animals have been selected for high and low "stress," high and low "analgesia," or both? That is, do HA mice display high levels of swim SIA because their endogenous pain inhibition systems are functionally upregulated or because they are simply experiencing more stress from the same stressor? The latter possibility is quite difficult to evaluate unambiguously, but we have obtained some evidence that HA/LA mice do respond differentially to swim stress. HA mice are poorer swimmers than are LA mice (B.S., unpublished data) and HA mice become more hypothermic after swims than do LA mice (102). Although these lines manifest equal basal metabolic rates, HA mice display about 30% lower oxygen consumption during swims in 20°C water (103), which may explain their greater swim hypothermia. Differential swim hypothermia is not sufficient to explain the differential SIA, however. After swimming in 38°C water, which produces no change in core body temperature in either line, HA mice still manifest greater swim SIA (102). Also, in a recently completed study, HA/LA mice exposed to cold ambient temperatures (−5°C to 1°C for 15 min) exhibited differential analgesia but equal hypothermia (B.S., unpublished data). Preliminary evidence obtained by using the open field and emergence neophobia tasks indicates that HA mice may be more emotionally reactive than LA mice (104). Thus, HA mice appear to experience swimming as a more severe stressor than do LA mice.

Nonetheless, overwhelming evidence exists that genes specifically underlying opioid (and nonopioid) mechanisms of endogenous pain inhibition have also been altered by selection. SIA in HA but not LA mice is attenuated by naloxone (89, 90, 96, 101), which has no effect on swim hypothermia (102). Adrenalectomy increases SIA magnitude in HA but not LA mice, and this effect is reversed by dexamethasone, supporting the hypothesis that the pituitary/adrenal axis has been altered in these animals (105). The administration of D-amino acids (enkephalinase inhibitors) facilitates SIA in HA but not in LA mice (106). In addition to SIA differences, HA mice manifest more analgesia from opiate agonists and electrical stimulation, as described above, in which the stress axis may be largely bypassed. Finally, we have recently demonstrated that HA mice display upregulated μ-opiate receptor-binding density (B_{max}) in whole-brain homogenates relative to LA mice and a particularly large (128%) increase in the medial thalamus (107). Thalamic μ receptors may play an important role in ascending pain inhibitory circuitry, and we have hypothesized that this subpopulation of receptors may comprise a genetically modifiable magnitude control mechanism for pain inhibition (107). Another relevant subpopulation may be in the nucleus raphé magnus, which displays higher μ receptor mRNA levels in HA versus LA mice (93).

Studies such as those described above are very useful for determining the effects of genetic alterations caused by selection, but they may be of little value for determining which genes have been altered. We have taken a first step toward this latter aim by investigating the mode of inheritance of swim SIA and opiate agonist-induced analgesia in HA/LA mice. Using

standard Mendelian breeding protocols, test crosses were set up using 27th-generation HA and LA mice. Genetically segregating backcross mice [HA × (HA × LA), LA × (HA × LA), and their reciprocals] and F_2 hybrid mice [(HA × LA) × (HA × LA) and reciprocals] were tested for opioid swim SIA (3-min, 38°C water), morphine analgesia (10 mg/kg, i.p.), and U-50,488H analgesia (30 mg/kg, i.p.) on the hot-plate test (108). Data obtained from such offspring can be used to estimate the number of independent genetic loci mediating the differential phenotypes of the parental lines (11). To our surprise, we found that the differential HA/LA responsivity to all three types of analgesia was determined oligogenically by one or at most two gene loci. The phenotypes did not show cosegregation, indicating that three distinct oligogenic effects were identified (108). It is important to note that these data do not necessarily imply that these complex, quantitative traits have simple genetic determination, but rather that the high levels of analgesia displayed by HA mice are due to alterations in a small number of genetic loci. Indeed, in a separate experiment we showed that 20°C-swim SIA magnitude is mediated polygenically by more than three genes (109). We believe that 38°C-swim SIA, morphine analgesia, and U-50,488H analgesia are probably affected by small subsets of those genes altered by the HA/LA selection. The utility of identifying correlated traits with large phenotypic divergence and oligogenic determination is that such traits are relatively easily mapped onto the mouse genome (14). We have stored tissue samples from each mouse used in the Mendelian experiment (108) and are presently mapping each phenotype by a search for linkage to polymorphic DNA markers (see below).

High Analgesic Response (HAR)/Low Analgesic Response (LAR) Mice. These mice were selectively bred in our laboratory for differing analgesic responsivity to the morphine congener levorphanol (110). Starting from a founder population of genetically heterogeneous stock (Binghamton HET), mice were tested for hot-plate (52.5°C) sensitivity following a single dose of levorphanol tartrate (1.6 mg/kg) and then retested 2 days later following a saline injection. Analgesia was defined as the ratio of levorphanol-treated to saline-treated latencies, and mice in the highest- and lowest-scoring quartiles were intermated to form the HAR and LAR lines (110). A randomly bred control line was maintained concurrently. In each successive generation, levorphanol doses were chosen to produce equipotent analgesia in each line to facilitate the identification of extreme responders. Significant line differences were evident by the third selected generation, and the realized heritability was estimated as $h_2 = 0.32$.

The HAR/LAR lines have been used largely to identify compounds sharing similarity in drug action with levorphanol; that is, to identify correlated pharmacological responses. In one study, dose–response relationships in HAR and LAR mice were evaluated for a number of analgesic compounds, and the slopes of the dose–response curves were compared. HAR to LAR slope ratios were observed in the following rank order: morphine > levorphanol > pentazocine > ethylketocyclazocine > U50,488H > clonidine (111). In a similar study, this rank ordering was observed: DAMGO > DADLE > [D-Ser^2,Leu^5]enkephalinyl-Thr (DSLET) > DPDPE (83, 112). These data sets thus reveal that selection has predominantly affected μ- over κ-opioid (111), δ-opioid (83, 112), and non-opioid (111) receptor mechanisms.

No differences in opiate pharmacokinetics were seen between HAR and LAR mice, and whole-brain-homogenate binding revealed no meaningful line differences (111). However, quantitative autoradiographic studies using [^{3}H]DAMGO revealed large (nearly 2-fold) differences in the dorsal raphe nucleus (DRN) (113), a brain area known to be involved in nociceptive processing (8). There are anatomical connections between the DRN and the medial thalamus (the brain area displaying highly differential μ-receptor density in HA vs. LA mice; see above), and DRN stimulation can inhibit nociceptive units in the medial thalamus (114, 115). Thus, we have suggested that the HA/LA and HAR/LAR selection projects may have genetically altered the same analgesic magnitude control pathway at different levels of the neuraxis (107).

Since the central nervous system mechanisms mediating endogenous pain inhibition are thought also to underlie opiate analgesia, we have investigated whether similar genes have been altered in the HA/LA and HAR/LAR projects. One study provided evidence for strong genetic commonalities, as HA/HAR and LA/LAR mice displayed both high and low swim SIA and levorphanol analgesia, respectively (92). Also, in a recently performed Mendelian segregation analysis of morphine analgesia in HAR/LAR mice, evidence for oligogenic determination of this phenotype was obtained (116). However, in this same study, a complementation analysis was performed between the recessive homozygotes of each selection project, HA and HAR mice. If the same one or two genetic loci were responsible for the high morphine analgesia displayed by these two lines, all HA × HAR offspring should exhibit this phenotype. Instead, a puzzling bimodal distribution of responses was observed, suggesting that different genes (with similar effects) have been fixed by these two selections (116). As with HA/LA mice, we are presently mapping genes underlying the HAR/LAR divergence. A comparison of the results of these two mapping efforts should reveal whether any overlap truly exists in their genetic determination.

High Autotomy/Low Autotomy Rats. One other selection project of particular interest to pain researchers was conducted by Devor and Raber (117) for high and low autotomy. Autotomy refers to self-injury to a denervated limb and may be an animal model of human deafferentation pain (118). Even when the nerve injury is similar, deafferentation pain in humans and autotomy in rodents show great individual variability in their expression. Starting with Sabra strain (Wistar) rats, these investigators selected for high and low autotomy scores following the unilateral sectioning of the sciatic and saphenous nerves (117). After 10–12 generations of successful selection, Mendelian test crosses were made as described above, and the data strongly suggested that propensity to autotomize is inherited through a single major recessive gene. Unoperated rats of these two selection lines have been found to differ in their responsivity to both mechanical and thermal nociceptive stimuli (119), suggesting that the "autotomy gene" may have general relevance to nociceptive processing.

B6, D2, and BXD RI Mice. A number of inbred mouse strains, including B6, BALB/c, A, C3H/He, and D2, have been utilized to study pain-relevant responses. Marked differences have been observed between strains with respect to the following: baseline latencies as measured on the hot-plate and tail-flick tests; autotomy; and analgesia from morphine, butorphanol, and buprenorphine, [Met]enkephalin and enkephalin analogues, D-amino acids, κ-opiate receptor selective agonists, nitrous oxide, naloxone, and conspecific defeat (see refs. 34, 83, and 120 for reviews; also see refs. 53, 54, 87, 121, 122, and 123). In general, the biggest differences in opiate analgesic magnitude exist between the D2 strain (high) and the B6 strain (low). In fact, these two strains differ on a myriad of responses to administered or stress-released opioids, including hypothermia (D2 > B6), antidiuresis (D2 > B6), constipation (B6 > D2), muscular rigidity (Straub tail; B6 > D2), behavioral activation (B6 > D2), morphine consumption (B6 > D2), learning/memory (B6 improved, D2 impaired), and physical dependence (B6 > D2) (34, 83, 120). The divergent origins of these two strains (29), with concomitant allelic differences in a large percentage of genes, partly explains the phenotypic differences. Unfortunately, the vast number of documented neuroanatomical, neurochemical, and neurophysiological differences between these strains (124) make it virtually impos-

sible to use the correlated-trait approach to explain their differential analgesic sensitivities.

Since gene mapping efforts are most fruitful when large progenitor-strain differences are demonstrated, the BXD RI set derived from B6 and D2 mice is particularly attractive for opioid research. RI strain panels were originally developed as a tool in detecting and mapping single gene loci with major effects on a trait of interest (31, 42), but theoretical advances (125, 126) and the wide availability of easily genotyped, polymorphic DNA markers of known location (15) have further permitted the provisional mapping of QTLs, each with only modest impact on the trait. In QTL mapping, one seeks to discover statistically significant associations between a quantitative trait and allelic variation at one or more previously mapped DNA marker loci, most commonly polymorphic simple sequence repeats (SSRs, or "microsatellites") that appear randomly throughout the genome. RI strains are particularly useful for QTL mapping because, through many years of cumulative effort, over 1500 microsatellite markers have been genotyped in each strain. As a practical matter, one simply needs to obtain phenotypic means for each strain in an RI panel and then correlate the strain distribution with the known allelic pattern of each marker. Because of the statistical power limitation imposed by the finite number of available RI strains (26 in the BXD series), genomic regions of putative statistical association usually need to be confirmed by using standard (B6 × D2)F_2 crosses. In the first such QTL mapping effort aimed at a pain-related phenotype, we (82, 127) have mapped morphine analgesic sensitivity in the BXD RI series and (B6 × D2)F_2 mice. The QTL accounting for the largest percentage of phenotypic variance between B6 and D2 mice (28–33%) was mapped to proximal chromosome 10 near the marker *D10Mit51*. Mice inheriting the D2 allele display 4-fold greater analgesia from morphine (16 mg/kg, i.p.) than mice inheriting the B6 allele (82). Interestingly (although not surprisingly), the gene encoding the murine μ-opiate receptor, *Oprm*, was recently mapped to this same chromosomal region (128), establishing *Oprm* as an obvious candidate gene for this QTL. A related phenotype, [^{3}H]naloxone-binding density (B_{max}), also maps to this region, suggesting that the sequence differences between D2 and B6 alleles may be found in a promoter or enhancer region affecting gene expression (82).

This mapping study also identified another putative QTL affecting morphine analgesia on chromosome 9 near *Ctl2*. A candidate gene in this region is *Htr1b*, which encodes the serotonin-1B receptor, and we are collecting converging lines of evidence pointing to the importance of this receptor subtype in opiate-analgesic magnitude (129). This finding illustrates the heuristic value of gene-mapping efforts, as the role of serotonin-1B receptors in analgesia has remained largely undefined due to the lack of specific pharmacological tools.

POMCX*4 Targeted Mutants. These mice represent the first example of the use of transgenic technology to "knock out" a pain-relevant gene. A gene-targeting vector, POMCX*4, was constructed in which the tyrosine codon at position 179 of the proopiomelanocortin (POMC) gene was converted by targeted mutagenesis to a premature translational stop codon (130). The resultant knockout mice express a truncated POMC prohormone, and although other POMC products—e.g., corticotropin and α-melanocyte-stimulating hormone) are produced normally in these animals, β-endorphin cannot be detected (131). Although these mice display no overt developmental or behavioral alterations and exhibit unchanged morphine analgesic sensitivity, they appear to be devoid of opioid-mediated swim SIA. Intriguingly, they also display upregulated nonopioid swim SIA and paradoxical naloxone-induced analgesia, both of which may reflect compensatory responses to the lack of β-endorphin (131). Projects are underway to produce knockout mice lacking functional proenkephalin and prodynorphin genes and the *Mor, Dor*, and *Kor* genes encoding the murine μ, δ and κ receptors, respectively (M. J. Low and D. K. Grandy, personal communication). Other existing transgenic models of possible relevance to nociceptive processing include *Htr1b* knockout mice (132) and mice lacking the α-subunit of the G_{i2} protein (133).

Genetic Mediation Is Specific to the Nociceptive Assay

Because of the large number of subjects that are usually required for genetic investigations, the vast majority of such research has been performed by using the easily employed hot-plate and/or tail-flick tests of nociception. Although these acute, thermal assays are poorly reflective of clinically important human pain states, they do predict analgesic effectiveness fairly well (134). However, considerable evidence suggests that different types of nociception are mediated by separable physiological mechanisms (see, for example, ref. 135), and, thus, one might expect the genetic mediation of sensitivity to these pain modalities to differ as well. Indeed, we have recently demonstrated that although HA, HAR, and D2 mice display longer hot-plate and tail-flick latencies (indicative of lower sensitivity) compared with LA, LAR, and B6 mice, respectively, there are no differences among these strains in nociceptive sensitivity to intraperitoneal injection of acetic acid (87).

In addition, evidence is accumulating that genetic factors underlying sensitivity to opiate inhibition of different pain modalities are similarly divergent. In the seminal work using the CXB RI series, the high opiate-receptor-density CXBH strain was found to be the highest scoring RI strain on the hot-plate test (49, 136) but was later found to exhibit merely average morphine sensitivity on the tail-flick test (46, 58). Also, on the hot-plate test, BALB/c mice were found to be the most sensitive strain (136–138), whereas, on the tail-flick test, B6 mice were more sensitive (46, 136). Some investigators have found no difference in morphine analgesic sensitivity between D2 and B6 mice on the acetic acid abdominal-constriction assay (53, 139). We have recently completed a study investigating morphine dose–response relationships in D2, B6, HA, LA, HAR, LAR, and CXBK mice on the four most common murine algesiometric assays, the hot-plate, tail-flick/withdrawal, acetic acid abdominal-constriction, and formalin tests (87). CXBK mice were found to display deficient sensitivity to morphine on all four assays compared with B6 mice. D2 mice were more sensitive to morphine than B6 mice on the hot-plate and tail-flick tests but not on the abdominal-constriction or the formalin test. No differences on either phase of the biphasic formalin test (see ref. 140) were found between HA and LA or between HAR and LAR mice. A surprising new finding is that by the abdominal-constriction assay LA and LAR mice were found to be significantly *more* sensitive to morphine analgesia than HA and HAR mice, respectively (87). We have no good explanation as yet for the inverse genetic correlation between morphine analgesia on the hot-plate/tail-flick versus abdominal-constriction assays in HA/LA and HAR/LAR mice. This finding, however, strongly emphasizes that genetic sensitivity to opiate analgesia can be highly specific to the nociceptive assay employed. These data point to the need for new, more clinically relevant genetic models to be identified or developed.

Conclusions and Clinical Relevance

The existence of a number of genetic models displaying large divergence in analgesic sensitivity clearly illustrates that these traits are heritable and amenable to study. The characterization of these models is encouraged by the repeated demonstration that only relatively simple genetic alterations—i.e., the effects of a few genes—are sufficient to produce the extreme responses of HA/LA, HAR/LAR, and CXBK mice (83, 108,

116). However, it is equally clear that the similar phenotypes of LA, LAR, and CXBK mice, for example, are produced by differential genetic alterations. The observation that different genetic mechanisms underlie opiate sensitivity for different nociceptive modalities further complicates the task at hand. In fact, one major conclusion derived from an analysis of the aforementioned genetic models is that additional models are undoubtedly required if we are to advance our knowledge significantly. For example, the analysis of a genetic model of chronic pain might be well worth the considerable effort required.

Thus far, the only confirmed relationship between a murine gene and a pain-related trait is for POMC and opioid SIA, respectively (see above; ref. 131), since the absence of this gene in knockout mice is accompanied by the absence of the trait. The mediation of morphine analgesic magnitude by *Oprm* (encoding the murine μ receptor) and *Htr1b* (encoding the murine serotonin 1B receptor) is implied by QTL mapping data (82, 127, 129) but not yet confirmed. Genes with major effects on thermal nociceptive sensitivity, morphine analgesia, SIA, and autotomy are on the verge of being localized and identified. The relevance to humans of such gene-mapping efforts is enhanced by the extensive syntenic conservation (and linkage homology) of the mouse and human genomes; about 80% of the mouse genome is estimated to match conserved regions of the human genome (141). This fact suggests, for instance, that the QTL for morphine analgesia on mouse chromosome 10 has a counterpart on the long arm of human chromosome 6 (6q24-25), where the human μ-receptor gene (*OPRM*) is located.

Investigations into the genetic mediation of pain-related traits may ultimately have considerable clinical relevance. One day it might be possible to screen patients (for allelic forms of relevant major genes) to predict their analgesic sensitivity to morphine. Such knowledge would allow individual tailoring of morphine dose to optimize pain relief and minimize side effects. Alternatively, genetic investigations might facilitate the development of nonopioid therapeutic agents. Further in the future, gene therapy might one day be employed for chronic pain patients refractory to opiates.

More broadly, genetic differences in traits such as morphine analgesia are likely to reflect differences in endogenous opioid functioning. Opioid peptides have been implicated in the mediation of numerous physiological processes. The identification of genes relevant to opioid analgesia is likely, therefore, to contribute to our understanding of many clinically relevant conditions beyond pain inhibition, such as immune modulation, natural reward states, and drug abuse.

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