ROLE OF THE H-2 SYSTEM IN REGULATING THE SENSITIVITY OF MICE TO MORPHINE

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The sensitivity to pain of mice of congenitally resistant (CR) strains based on A/Sn, C3H/Sn, and C57BL/10/Sn strains was studied after injection of morphine into the animals. Among the CR strains based on A/Sn and C3H/Sn, strains sensitive and resistant to the narcotic were distinguished. Sensitivity to morphine is inherited as a dominant. The possibility of the participation of the principal H-2 histocompatibility system in mice in the genetic control of the sensitivity of mice to morphine is discussed.

KEY WORDS: morphine; sensitivity to pain; congenitally resistant strains.

Sensitivity to narcotics, determined on the basis of hypnotic and analgesic tests, tests of locomotor activity, and so on, varies sharply in mice of different strains and, consequently, is under genetic control. This type of pain sensitivity has been shown to have a dominant or codominant type of inheritance [4]. One difficulty of the problem is that control of sensitivity to narcotics is polygenic, and as yet no markers have been found for the identification of the individual genetic loci participating in this process [5]. The possibility that the sensitivity of mice to morphine is regulated by the principal H-2 histocompatibility system has been argued theoretically, for it incorporates loci which control the strength of the immune response to a whole series of antigens [1]. Many congenitally resistant (CR) strains of mice have now been bred for the study of the H-2 system; they differ from each other either with respect to the whole H-2 system or to certain of its loci, the remaining genes being identical.

The object of this investigation was to determine the degree of participation of H-2 systems in the genetic control of sensitivity of mice of CR strains to morphine.

EXPERIMENTAL METHOD

Male mice of CR strains obtained on the basis of strains A/Sn, C3H/Sn, and C57BL/Sn were used.

The mean weight of the experimental animals was 25 g. The mice were kept on an ordinary laboratory diet. The analgesic response to a single intraperitoneal injection of morphine sulfate in a dose of 20 mg/kg was determined 1 h after injection by the hot plate method (t = 55°C) [3]. In cases when the duration of the nociceptive response exceeded 1 min, observations on the animal were stopped and the time was considered to be 60 sec.

EXPERIMENTAL RESULTS

The experiments on standard inbred strains (Table 2) showed that strains A/Sn and C3H/SN were much more sensitive to morphine, according to the pain test, than C57BL/10 mice. Similar experiments, carried out on mice of CR strains based on C57BL/10 revealed no significant differences in the response to morphine as shown by the sensitivity to pain test. Meanwhile, among CR strains based on A/Sn and C3H/Sn, whose analgesic response was increased several times over after injection of morphine, strains differing in their sensitivity to the narcotic could be distinguished. For instance, the duration of the pain test to a thermal stimulus in ASA mice was increased by 474% compared with the control (a high response), whereas in A/Sn and ASW mice the increase was 386 and 294% respectively (intermediate and low responses). The term "low" response is used only to describe the intensity of the response to morphine among CR strains of one group,

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TABLE 1.

Strain	H-2 haplotype
A/SnY	$H\text{-}2^a$
ASw	$H\text{-}2^s$
A. Ca	$H\text{-}2^f$
C3H/SnY	$H\text{-}2^k$
C3H.OH	$H\text{-}2^o$
C3H.YK	$H\text{-}2^{ja}$
C3H.SW	$H\text{-}2^b$
C3H.NB	$H\text{-}2^p$
C57BL/10 SnY	$H\text{-}2^b$
R 101	$H\text{-}2^g$
R 103	$H\text{-}2^g$
B10.CNB	$H\text{-}2^p$
B10.M	$H\text{-}2^f$
B10.D2	$H\text{-}2^d$

TABLE 2. Effect of Morphine on Sensitivity to Pain of Mice of CR Strains and F_1 Hybrids

Strain of mice	Group	Number of mice	Mean weight	Sensitivity to pain		Change, in %
				control	experiment	
A/Sn	1-	8	26,2	10,9±1,06	42,1±5,32	386
A·CA		10	22,1	10,0±0,41	47,4±4,08	474
A·SW		9	22,8	9,0±0,45	26,5±2,8	294
C3H/Sn	2-	12	30,8	11,6±0,34	35,0±3,45	301
C3H·OH		7	28,5	14,1±1,12	57,1±1,43	425
C3H·NB		6	27,8	14,7±1,0	54,3±3,12	408
C3H·SW		8	26,0	12,8±0,79	32,8±0,24	256
C3H·IK		7	24,8	12,6±0,39	46,0±4,91	365
C57BL/10 Sn	3-	11	21,6	10,5±0,59	13,7±0,86	130
B10·D2		7	24,4	9,0±0,54	11,4±0,61	126
B10. M.		6	24,6	11,6±0,62	13,6±0,55	117
B10. CNB		6	26,7	9,8±0,63	12,0±0,54	122
R 103		6	20,9	8,6±0,50	9,2±0,70	107
R 101		7	25,8	10,0±0,36	10,5±0,87	101
(A. CA×ASW) F_1	4-	7	16,7	11,0±1,32	45,1±0,51	410
(C3H. OH×C3H/Sn) F_1		6	23,1	13,6±0,81	54,6±2,9	415
C3H. NB× C3H. SW) F_1		8	15,8	14,6±0,77	51,4±0,84	351

for only strains based on C57BL/10 were absolutely resistant to the narcotic among all the strains tested. A high response to morphine in the second group, as shown by the result of the pain sensitivity test, was shown by mice of the C3H·OH and C3H·NB strains (425 and 408% respectively) and intermediate and low responses were observed in mice of strains G3H·IK (365%), C3H·SN (301%), and C3H·SW (256%). Since mice of each of these groups differed only with respect to their H-2 system of genes and adjacent regions of the chromosome, one of the loci determining sensitivity to morphine evidently must lie in the H-2 complex or be closely linked with it. In C57BL/10 mice, genetic determinants not linked with the H-2 locus evidently are responsible for the low sensitivity to the narcotic as revealed by the pain test. To discover the gene linked with H-2 it was therefore necessary to use other methods. Experiments on DBA, C57BL/6 and BALB/C mice in fact showed that locomotor activity and pain sensitivity are controlled by different genetic loci and that the responses of these strains to morphine, studied by the pain and locomotor tests, dissociate. For instance C57BL/6 mice, whose motor activity is increased several times over after injection of morphine, were found to be resistant to the analgesic effect of the narcotic. In DBA mice the opposite relationships were found [2]. The use of the locomotor test would probably reveal high and low responses of CR strains based on C57BL/6 and C57BL/10. However, for the immediate purpose the analgesic response was more adequate for the analysis of genetic mechanisms controlling sensitivity or resistance to morphine, for the mechanism of formation of opium addition evidently includes conformational changes in the brain receptors participating, in conjunction with endorphines, in the transmission of the nociceptive impulse in the intact organism [6].

To determine the inheritance of the sensitivity of mice to morphine, F_1 hybrids obtained by crossing mice of high- and low-responding strains based on A/Sn and C3H/Sn were used (Table 2). The response of all the hybrids was found to be similar to that of the strongly responding parental strains. These findings are evidence of the dominant type of inheritance of high sensitivity to morphine, as determined by the analgesic test.

The results are of practical importance in connection with the promising use of HLA antigens (the human histocompatibility system) as markers of individual sensitivity to opiates.

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ACTION OF IONOPHORE A23187 ON THE STRENGTH OF CONTRACTION AND TRANSMEMBRANE ACTION POTENTIAL OF GUINEA PIG PAPILLARY MUSCLE

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The calcium ionophore A23187, in a concentration of 2.5 μM, caused a two- to threefold increase in the strength of the contraction of the isolated papillary muscle of the guinea pig heart, stimulated at a frequency of 0.2 Hz. The ionophore A23187 reduced the resting voltage of the preparation in the intertrial interval and reduced the total duration of contraction. The increase in the strength of contraction was not accompanied by any change in the amplitude or duration of the transmembrane action potential. In the presence of the ionophore the ascending phase of a single contraction cycle showed a discontinuity separating the development of the twitch into two phases; differentiation of the twitch gave two positive maxima. The substance D-600, which blocks the calcium current, reduced the duration of the action potential and inhibited the second phase of twitch development, but caused no change in the magnitude or rate of the first phase of contraction. It is suggested that under the influence of the ionophore the component of the twitch which is not blocked by D-600 is caused by liberation of calcium from the sarcoplasmic reticulum.

KEY WORDS: ionophore; calcium; twitch; action potential.

The carboxyl antibiotic A23187, which has the property of forming electrically neutral complexes with bivalent cations, acts as a carrier of Ca^{2+} through the cell membrane [5]. In the first investigations to study the effect of this ionophore on the myocardium, its positive inotropic action was not found [6]. Later, however, the ionophore A23187 was shown to increase the strength of myocardial contraction in warm-blooded animals, evidence of an increase in the intracellular calcium concentration in the presence of the ionophore [3, 4]. On the other hand, data on the effect of A23187 on the duration and level of the plateau phase of the transmembrane action potential of isolated Purkinje fibers were given in [2].

It was thus not decided whether the positive inotropic action of ionophore A23187 is connected with changes in the electrical activity of the myocardial cells. The object of this investigation was to study that problem.

EXPERIMENTAL METHOD

Experiments were carried out on isolated papillary muscles from the hearts of guinea pigs (300-350 g). The papillary muscle was isolated from the left or right ventricle and placed in a transparent plastic chamber

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