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High Activity of Potent Analgesics on Conditioned Rat Tranquilizer Test. (25029)

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Inhibition of conditioned avoidance-escape response in rats has been widely used as one of the pharmacological tests for tranquilizing agents of reserpine and chlorpromazine types (1,2). Furthermore, it has been reported that blocking of this conditioned response is also produced by morphine(1). To investigate the possible correlation between analgesic potency and inhibition of this conditioned response with compounds related to morphine, we have now compared 3 analgesics and 2 well known tranquilizers on this test. The 2 tranquilizers used were chlorpromazine hydrochloride (Thorazine Hydrochloride ampuls, 25 mg/cc) and perphenazine (Trilafon ampuls, 5 mg/cc). The 3 analgesics were the following: Codeine Phosphate, U.S.P., a moderately potent analgesic; Morphine Sulfate, U.S.P., a highly potent analgesic; and *l*-14-hydroxydihydromorphinone hydrochloride (HDM hydrochloride, Numorphan Hydrochloride), a new and very highly potent analgesic. The latter has been reported 12 to 18 times as active as morphine sulfate by injection in mouse analgesia studies(3,4) and 8 to 10 times as active in clinical studies(3,5,6,7).

Methods. The apparatus and method for conditioned avoidance-escape response were essentially those described by Cook and Weidley(1). Rats were conditioned to jump onto a wooden pole at the sound of a buzzer to avoid a mild electric shock from metal rod floor. If the conditioned response to buzzer was blocked, the electric shock was then turned on to make the rat jump to the pole by unconditioned response. This procedure demonstrated that blocking was specifically of the conditioned response and was not attributable to general depression or motor weakness. Female albino rats of CF strain (Carworth Farms), weighing 120-220 g, were used. In a confirmatory experiment full-grown female albino rats of a different strain (Supplee-Wistar), weighing 200-300 g, were used. Analgesic activity was determined by the mouse hot-plate method(8) in CF-1 male mice (Carworth Farms), weighing 20-28 g. The ED_{50} values were calculated graphically(9).

Results. In the first experiment on conditioned response, the 5 drugs were administered subcutaneously to CF rats, 40 rats being used for each drug. The 3 analgesics all demonstrated blocking activity on this test, although of varying degrees (Table I). The very highly potent analgesic HDM was even slightly more active than perphenazine, one of the most potent tranquilizers in clinical use. HDM was about 1.6 times as potent as perphenazine on this test, calculated as base. Essentially similar results were obtained when the 2 compounds were compared again in older rats of the Supplee-Wistar strain, although the

TABLE I. Inhibition of Conditioned Response, by Subcutaneous Injection.

Drug	$ED_{50} \pm$ S.E.,* mg/kg	Potency
Chlorpromazine	7.0 ± .7	1.0
Perphenazine	.42 ± .05	17
Codeine	49 ±7	.14
Morphine	7.4 ± .8	.95
HDM	.26 ± .01	27

* Calculated as base.

TABLE II. Comparative Potencies on Conditioned Response Blocking and Analgesia Tests, by Subcutaneous Injection.

Drug	Conditioned response blocking		Analgesia		
			Mouse		
	$ED_{50} \pm$ S.E., mg/kg	Potency	$ED_{50} \pm$ S.E., mg/kg	Potency	Man potency
Codeine phosphate	70 ± 9	.14	18 ± 2	.12	.15
Morphine sulfate	9.9 ± 1.0	1.0	2.2 ± .4	1.0	1.0
HDM hydrochloride	.31 ± .01	32	.12 ± .02	18	10

latter were more sensitive and showed blockage of conditioned response at lower dosage baseline. In this confirmatory experiment values for blocking $ED_{50} \pm$ S.E. were perphenazine 0.21 ± 0.02 and HDM 0.16 ± 0.02 mg/kg, HDM again appearing slightly more potent than perphenazine.

The patterns of action differed somewhat in that the analgesics showed a more rapid onset of blocking than did the tranquilizers. Average times of onset at approximately the ED_{50} were as follows: chlorpromazine 114, perphenazine 141, codeine 36, morphine 32, and HDM 39 minutes. However, average duration of action of the tranquilizers was longer than that of the analgesics, as follows: chlorpromazine 276, perphenazine 246, codeine 152, morphine 140, and HDM 141 minutes.

Analgesic activities of codeine phosphate, morphine sulfate, and HDM hydrochloride were determined in mice, using 80 mice for each drug. In Table II the 3 analgesics are compared for potency by subcutaneous administration in the conditioned response blocking test, in mouse analgesia, and with reported figures for analgesia in man(3,6,10). As the clinical studies cited were always carried out in comparison with morphine sulfate dosage, potency values in the Table have been calculated as weights of salts and compared with morphine sulfate as 1. There is a rough correlation between rat conditioned response blocking potency and both mouse and human analgesic potencies. Thus, on the 3 different tests codeine phosphate had only 0.12-0.15 of the potency of morphine sulfate, whereas HDM hydrochloride was 10-32 times as potent as morphine sulfate. Parenthetically, chlorpromazine and perphenazine are not considered to produce analgesia, although they do potentiate analgesics, *e.g.*, morphine. Unlike the 3 analgesics, in the mouse hot-plate test chlorpromazine and perphenazine showed an analgesic response only at dosages which also produced obvious sedation and central nervous system depression.

When HDM was administered orally, its potency on mouse analgesia test was greatly reduced, being only 0.07 of its subcutaneous potency. Similarly, its oral potency on conditioned response blocking test was also markedly reduced to about 0.02 of its subcutaneous potency, whereas oral potency of the tranquilizer, perphenazine, was reduced much less, *i.e.*, only to about 0.2 of its subcutaneous potency. Thus, although HDM was about 1.6 times as active as perphenazine on the conditioned rat test subcutaneously, it was only 0.1 as active orally.

Activity in the conditioned response blocking test was also shown by synthetic narcotic analgesics, such as meperidine. However, sodium salicylate appeared inactive both subcutaneously and orally.

Although the significance of high "tranquilization" test activity of potent analgesics is conjectural, it is interesting that a rough correlation between conditioned response blocking activity and analgesic activity was found in these 3 analgesics of widely differing potencies. This tranquilizer-like action may be related to the hypothesis advanced(11) that reduction of pain-anticipatory anxiety is a necessary action that a drug must exert to be a potent analgesic.

Summary. The analgesics codeine, morphine, and *l*-14-hydroxydihydromorphinone (Numorphan) produced inhibition of the conditioned avoidance-escape response in rats, a widely used tranquilizer test. The potencies in blocking conditioned response correlated

roughly with analgesic potencies. The very potent analgesic, *l*-14-hydroxydihydromorphinone, exhibited by subcutaneous injection slightly more conditioned response blocking activity than perphenazine, one of the most active tranquilizers now in clinical use.

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Induction of Immunological Tolerance to Male Skin Isografts in Female Mice Subsequent to Neonatal Period.* (25030)

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Histo-incompatibility to male skin isografts in females of some inbred strains of mice, particularly those of A and C_{57}Bl strains, was first reported by Eichwald and Silmser(1) and later confirmed by others(2-8). Some investigators believe that this phenomenon occurs in most strains(6,9). Eichwald *et al.*(10), Hauschka(11), and Snell(12) have interpreted rejection of male skin by female of same strain as an immune response due to recipient's lack of donor's isoantigen which originates from a gene on the Y chromosome. The immunological nature of this phenomenon has been further supported by recent findings of Billingham *et al.*(4) and Mariani *et al.*(5) which demonstrate that induction of acquired tolerance to overcome the histo-incompatibility to male skin isografts, can be produced in females of specific strains by injecting them at birth with viable spleen cells obtained from isologous adult male donors. Our findings indicated that the age of both recipient and donor at time of skin isotransplantation influences the frequency of male skin rejection by females of same inbred strain (19). In essence, these investigations demonstrated that weanling females would more frequently accept male skin than would older females of the same strain. As a result 2 studies were undertaken: (1) To determine the possibility of inducing a state of tolerance in female mice of A and C_{57}Bl (subline 1) strains by I. V. injection of viable male spleen cells during post neonatal period and (2) to determine whether tolerance to male skin could also be induced in A and C_{57}Bl (subline 1) females by joining them in parabiosis with isologous males.

Method. In a first series of experiments 2 groups of A and C_{57}Bl (subline 1) strains of mice pre-treated with spleen cells were used. Spleen cells were prepared according to method previously described(18,20). Approximately 20 million viable male spleen cells suspended in 0.2 cc of Ringer-Locke's saline solution were injected intravenously into the tail vein of A females 30-47 days old and 92 days old recipients. Cell viability was determined by the eosin method(21). Similar numbers of spleen cells/mouse were also transferred from isologous adult C_{57}Bl (subline 1) males to C_{57}Bl (subline 1) females of 32-48

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