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A Study of the Shaking and Grooming Induced by RX 336-M in Rats

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GMEREK, D. E. AND A. COWAN. *A study of the shaking and grooming induced by RX 336-M in rats.* PHARMAC. BIOCHEM. BEHAV. **16**(6) 929–932, 1982.—Several endogenous peptides and experimental agents induce "wet-dog" shakes and excessive grooming after acute administration to rats, but quantitative information on a possible relationship between the two behaviors is lacking. RX 336-M (7,8-dihydro-5',6'-dimethylcyclohex-5'-eno-1',2',8',14 codeinone) is a novel compound which elicits dose-related shaking and grooming in the rat. We have measured and compared the shaking and grooming induced by several doses of RX 336-M (1.5–12 mg/kg, IP) in male Sprague Dawley rats at various stages of maturation. Analysis of the correlation between the number of "wet-dog" shakes and the frequency of grooming episodes indicates that a relationship may exist between the shaking and grooming. The excessive grooming induced by RX 336-M may be a mechanism by which the rat's state of arousal (raised by the shaking) is lowered and homeostasis is maintained.

Grooming "Wet-dog" shakes Quasi-morphine withdrawal syndrome Arousal Homeostasis

ACUTE peripheral administration of RX 336-M (7,8-dihydro-5'-6'-dimethylcyclohex-5'-eno-1',2',8',14 codeinone) to drug-naive rats causes a syndrome consisting of behavioral activation, rapid forepaw movements, excessive grooming, and whole body "wet-dog" shakes (WDS) (rapid twisting of the head and trunk with the forepaws leaving the ground) [9]. These behaviors are most often observed in morphine-dependent rats undergoing withdrawal or precipitated abstinence. RX 336-M (Fig. 1) has been studied as a prototype inducer of this so-called quasi-morphine withdrawal syndrome (QMWS) [8]. Thyrotropin releasing hormone (TRH) [15,23], methylxanthines [7] and sodium valproate [9,10] are further examples of compounds capable of eliciting a QMWS. Several neuropeptides induce WDS and/or excessive grooming when given centrally to mice or rats, including $ACTH_{1-24}$ [12], beta-endorphin [13], bombesin [5], and substance P [20]. Why drug-naive animals should display WDS and excessive grooming in response to a range of endogenous and exogenous agents is, as yet, unknown.

Our finding that RX 336-M induces both shaking and excessive grooming in a dose-related manner [14] has afforded us the unique opportunity of studying these behaviors together, in the same animal. During the course of our work, we noticed that RX 336-M is much more effective in producing WDS and grooming in immature rats than in older rats. In the present experiments, we have investigated the relationship between the shaking and grooming induced by RX 336-M in both young and mature rats. We conclude that the extent of grooming is closely related to the incidence of shaking induced by this experimental compound.

METHOD

Male Sprague Dawley albino rats (Zivic-Miller) were housed in groups of 6 at 23±1°C with food and water provided ad lib. A standard light-dark cycle was maintained with a timer-regulated light period from 0600 hr to 1900 hr.

Rats were placed singly in Plexiglas observation boxes (22 cm long; 18 cm wide; 25 cm high) and allowed to habituate to the new environment for at least 15 min. Experiments were performed between 1300 and 1700 hr. The shaking and grooming of 4 animals was monitored simultaneously for 30 min starting immediately after the last injection of RX 336-M. The total number of WDS displayed was counted for each rat throughout the observation session. Grooming was scored in an intermittent fashion, 5 sec out of every 20 sec, with a portable microcomputer [21]. A positive grooming score was given if any element of grooming (e.g., washing, scratching, biting) was displayed during any of each rat's 5 sec intervals. In this manner, there were 90 possible grooming scores for each rat. The results are presented as the percent of the maximum possible number of grooming episodes (%MGE). Groups of 6–10 animals were tested for each set of experimental conditions. Regression lines for %MGE (and the number of WDS) were determined by the method of least squares. The r-values were calculated with %MGE as the dependent variable. Statistical significance was evaluated by the Mann-Whitney U test.

Morphine sulfate (Mallinckrodt) and RX 336-M maleate (Reckitt and Colman) were dissolved in saline and given in volumes of 5 ml/kg, SC and 10 ml/kg, IP, respectively. Doses

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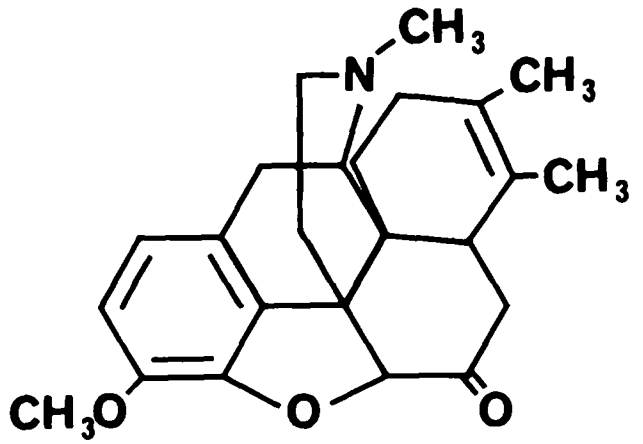

FIG. 1. RX 336-M.

of these compounds were calculated in terms of the particular salt.

RESULTS

The grooming induced by RX 336-M consists primarily of face washing and preening of the flanks with the mouth or forepaws. Very little scratching with the hindpaws occurs. Generally, there is a sequential nature to the behavior of rats given RX 336-M. The rat shakes, then washes his face, and progresses to grooming of the flanks; after which the sequence starts over. This pattern of behaviors (grooming occurring immediately after the WDS) has been noticed to take place with several other shake-inducing compounds, and during morphine withdrawal [8]. Whereas the grooming induced by RX 336-M appears to be patterned, it is much less stereotyped than the excessive scratching induced by central administration of bombesin, or the oral and facial stereotypies elicited by TRH (Gmerek and Cowan, unpublished observations). In fact, mature rats (which do not shake as often as immature rats) display grooming that is indistinguishable from that of untreated rats.

With immature rats (120–140 g), increasing the dose of RX 336-M from 1.5 mg/kg to 3 mg/kg, and then to 6 mg/kg, decreased the correlation between the number of WDS and the %MGE (Fig. 2). When rats in the weight range of 190–210 g and 280–300 g, respectively, were tested, the correlation improved correspondingly (Table 1); at a dose of 6 mg/kg of RX 336-M, the high r-value (0.96) in rats weighing 280–300 g is suggestive of a positive relationship between the shaking and grooming. Note, however, that the overall effectiveness of RX 336-M is less pronounced in these larger animals. While 6 mg/kg RX 336-M produced 96 WDS during 30 min in 120–140 g rats, only 18 WDS occurred in rats weighing 280–300 g. Doubling the dose of RX 336-M (to 12 mg/kg) in the large rats increased the number of WDS which, in turn, decreased the correlation. Note that while grooming also decreased in the more mature rats, the 280–300 g rats still groomed significantly more ($p=0.02$) after 6 mg/kg of RX 336-M than after saline.

The low r-values obtained with small rats or with high doses of RX 336-M is most likely due to the fact that the animals shake so often. The WDS dominate, and the time available for grooming between each shake becomes a limiting factor.

Morphine antagonizes both the shaking and grooming induced by RX 336-M [14]. We therefore pretreated immature rats with the narcotic (0.5 and 1 mg/kg, SC at −15 min) and found that, although the %MGE does decline, the correlation between shaking and grooming improves (Fig. 3).

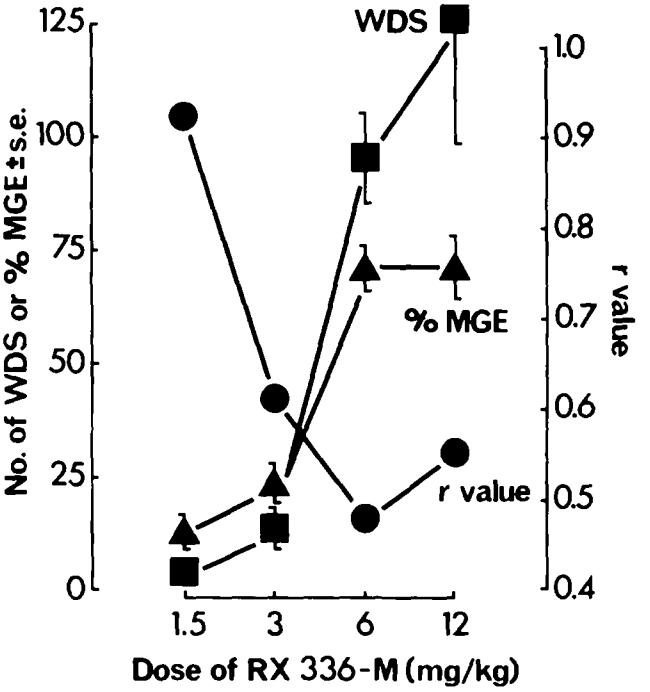

FIG. 2. Effect of increasing doses of RX 336-M, in rats weighing 120–140 g, on the number of WDS (■), the % MGE produced (▲), and the r-values (●).

TABLE 1

THE RELATIONSHIP BETWEEN SHAKING AND GROOMING INDUCED IN RATS BY RX 336-M

Wt. range (g)	N	No. of WDS (mean ± s.e.)	%MGE ± s.e.	r-Value*
80–90†	7	123 ± 8	72 ± 2	0.24
120–140†	8	96 ± 11	70 ± 3	0.48
190–210†	8	40 ± 9	37 ± 6	0.85
280–300†	8	18 ± 7	21 ± 6	0.96
280–300‡	8	61 ± 20	33 ± 9	0.87

*Values were determined by linear regression.
†RX 336-M (6 mg/kg, IP).
‡RX 336-M (12 mg/kg, IP).
Control (saline) %MGE scores for each of the four weight ranges were between 8 ± 2 (s.e.) and 9 ± 2 (n=6–8).

DISCUSSION

Several endogenous peptides (e.g., $ACTH_{1-24}$, TRH and beta-endorphin) [4, 13, 14, 15, 17], the antiepileptic agent

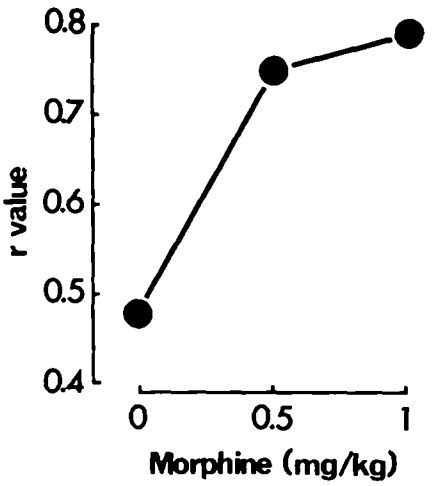

FIG. 3. Effect of increasing doses of morphine sulfate (15 min prior to 6 mg/kg RX 336-M, IP in 120–140 g rats) on the r-values (determined by linear regression on the number of WDS and % MGE).

sodium valproate [9,10], and at least 3 experimental compounds (AG-3-5, RX 336-M, Sgd 8473) [18,22], cause "wet-dog" shakes and excessive grooming after acute administration to rats. Although much has been published on these behaviors, the approach has uniformly been one of studying either shaking or grooming (i.e., separately) after chemical or surgical intervention. There is currently a lack of quantitative information on a possible relationship between shaking and excessive grooming when they are induced in the same animal. The present study, then, is concerned with the following simple yet unresolved question: why do rats shake and groom excessively in response to certain chemically-diverse compounds? In addressing this issue, we chose to use RX 336-M, a novel dihydrocodeinone that elicits dose-related shaking and grooming in the rat. After manipulating these behaviors by (a) using rats at different stages of maturation, and (b) pretreating the animals with morphine, we conclude that the extent of grooming is closely related to the incidence of shaking caused by RX 336-M.

Rats normally spend a good deal of their awake time grooming [3]. While grooming often serves merely as maintenance behavior, it also acts as a mechanism for heat dispersion in the rat [1]. RX 336-M causes a slight hyperthermia, but since tolerance develops to the grooming [14] but not to the hyperthermia [9] induced by RX 336-M, it is doubtful if the rat is grooming in order to spread saliva for evaporation and subsequent heat loss. Grooming also occurs when rats are stressed or placed in a conflict situation [16]. A common denominator to stress, anxiety and conflict situations is an increase in arousal. While it does not seem to be stress or anxiety which precipitates the behaviors observed with RX 336-M, this compound does appear to cause an increase in arousal. Berlyne [2] suggested that higher animals strive to maintain an intermediate level of arousal. Cortical steady (DC) potential data indicate that grooming is associated with a decrease in arousal [6]. Delius [11] therefore suggested that displacement grooming is the result of drug-, conflict- or stress-induced increases in arousal; and that such grooming is thus acting as a homeostatic mechanism for de-arousal. Jolles, Rompa-Barendregt and Gispen [19] proposed that excessive grooming is a secondary response, serving to de-arouse an activated animal.

There are situations (stress, heat) and compounds ($ACTH_{1-24}$, bombesin) which cause excessive grooming without a high incidence of shaking, but most situations associated with shaking (morphine withdrawal, wet fur), and many of the shake-inducing agents, also cause increased grooming in rats. Thus, we have found that the IP administration of AG-3-5 (0.8 mg/kg), Sgd 8473 (30 mg/kg), sodium valproate (300 mg/kg) or TRH (20 mg/kg) elicits marked increases in shaking and grooming; in the weight range of rats tested (120–140 g), the r values obtained were 0.64, 0.85, 0.96 and 0.85, respectively (Gmerek and Cowan, unpublished results).

The chronological relationship between the occurrence of shaking and grooming induced by RX 336-M (grooming occurs immediately after shaking when it is physically possible), and our inability to separate the grooming from the shaking (AD 50 values for morphine and for haloperidol are comparable for attenuation of shaking and grooming—Gmerek and Cowan, submitted), substantiates but, of course, does not prove our belief that these behaviors are intimately related. The correlation between the two behaviors also indicates that such a relationship may exist.

RX 336-M-induced behavioral activation and WDS may be indicative of arousal. Thus, the grooming observed in the present study may very well be a mechanism by which the rat's state of arousal, which has been raised by the shaking, is lowered and homeostasis is maintained.

ACKNOWLEDGEMENT

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