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Peptide-Induced Excessive Grooming in the Rat: The Role of Opiate Receptors

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ALOYO, V. J., B. SPRUIJT, H. ZWIERS AND W. H. GISPEN. *Peptide-induced excessive grooming in the rat: The role of opiate receptors*. PEPTIDES **4**(6) 833–836, 1983.—We have investigated the possibility that opiate peptides induce excessive grooming behavior in the rat via a direct action on an opiate receptor by comparing the opiate agonist dynorphin(1–13) with its non-opioid fragment des-tyrosine[1]-dynorphin(1–13) (dT-Dyn). We have shown that both peptides are capable of inducing grooming and that this behavior can be suppressed by pretreatment with naloxone. Analysis of the grooming pattern revealed that the response induced by dT-Dyn is qualitatively similar to that induced by ACTH(1–24) and dynorphin(1–13). Cross-tolerance was demonstrated among the various peptides. We conclude that peptide-opiate receptor interaction is not the primary event in the induction of grooming and that the opiate receptor(s) involved are located at another site underlying peptide-induced grooming.

Grooming behavior Dynorphin(1–13) β-Endorphin Des-tyrosine[1]-dynorphin ACTH Naloxone

INTRACRANIAL (IC) or intracerebroventricular (ICV) administration of a number of neuropeptides in rodents initiates a wide variety of behavioral responses, a common element of which is the display of grooming behavior (for reviews see [11,16]). Thus far, the best characterized grooming response is that seen after ICV administration of N-terminal fragments of ACTH (ACTH(1–24), ACTH(1–16)-NH_2; [11, 12, 16]). In the search for a possible central mechanism of action of ACTH and congeners, it was found that the interactions of ACTH and opiates with central nervous structures have certain features in common [1, 13, 17, 20]. Since it was shown that ACTH-, β-endorphin- and dynorphin(1–13)-induced grooming could be blocked by specific opiate antagonists [6, 9, 10, 23], the question arises whether this grooming originates from peptide opiate-receptor activation. Some of the available literature can be interpreted to indicate that such is indeed the case, however, the evidence is far from conclusive [11].

In order to investigate whether interaction with an opiate receptor is the primary event leading to excessive grooming behavior, we have studied the grooming-inducing potency of the opioid peptide dynorphin(1–13) and its non-opioid, des-tyrosine fragment. Removal of the N-terminal tyrosine from opioid peptides has been shown to reduce their affinity for the opiate receptor [5]. Therefore, the grooming-inducing activity of des-tyrosine[1]-dynorphin(1–13) (dT-Dyn) would imply that direct interaction with an opiate receptor is not likely to be the initial event leading to grooming.

METHOD

Animals

Male Wistar albino rats, weighing about 140 g were used as subjects. The rats were housed in a 12:12 light:dark cycle and tested in the light phase of the cycle with free assess to food and water. One week prior to the test, a polyethylene cannula (length 4.5 mm, diameter 0.8 mm) was implanted into the interventricular foramen of the brain ventricular system as described in detail by Brakkee *et al.* [3]. In all experiments naive rats were used.

Grooming Test

The behavioral procedure was essentially the same as described by Gispen and coworkers [8]. The peptides were dissolved in saline immediately prior to use. To initiate the grooming test, the conscious rats received an ICV injection of peptide (1 μg dissolved in 3 μl, except for β-endorphin: 0.3 μg in 3 μl) or saline (3 μl) and were then placed individually into a novel glass box (20×12.5×14 cm) in a sound-proof observation room. After 5 min of adaptation, the grooming behavior was scored every 15 sec for a period of 55 min, leading to a maximal possible score of 220. Thus, every 15 sec the observer scored whether or not a given rat displayed one of the following behavioral elements: head washing, body grooming, anogenital grooming or scratching/licking paw as grooming elements and a rest category included all other behavior [8]. In the first experiment every group consisted of 5 animals. Their total grooming scores are depicted in Fig. 1. For comparing the structure of the grooming bouts seen after adminstration of the various peptides—in these animals—the frequencies of the displayed grooming elements were calculated relative to the amount of grooming displayed per rat (Fig. 3).

In a second experiment—a so-called cross-tolerance design—a first grooming test at time point zero (T=0) is

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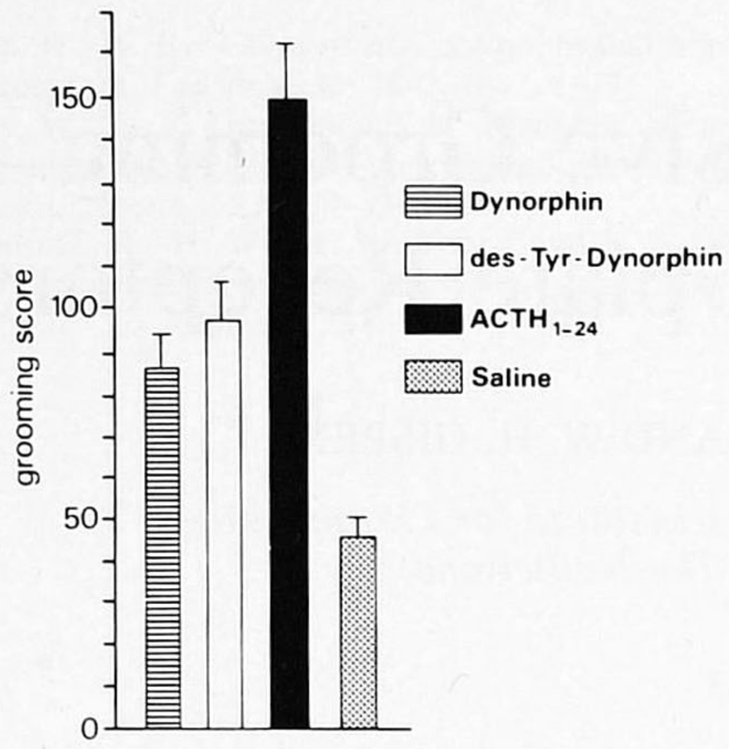

FIG. 1. The induction of excessive grooming by several peptides. The total grooming score (±SEM, n=5) elicited by dynorphin (1 μg), dT-Dyn (1 μg), ACTH(1–24) (1 μg) and saline are shown. Data are expressed as the total sum of grooming elements (maximum 220).

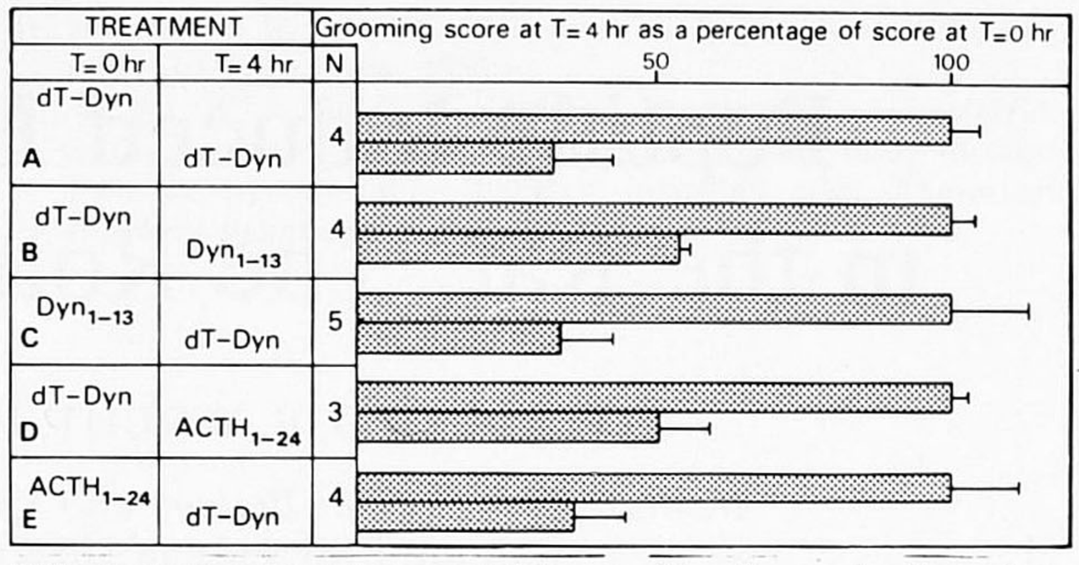

FIG. 2. Interaction among peptides resulting in a reduction of excessive grooming behavior. The grooming elicited by a second administration of peptide was compared to that elicited by a dose given 4 hr earlier and was presented as a relative score of the first score (calculated per animal). The dose of peptide administered was the same as that indicated in Fig. 1 except that 0.1 μg ACTH(1–24) was used. The grooming scores of the control groups (saline at T=0, peptide at T=4) exceeded the 100% at T=4 (not shown). A *t*-test with paired values was used to compare first treatment with the second treatment.

followed by a second test 4 hr later (T=4). All the cross-tolerance experiments consisted of a saline and an experimental group. The number of animals per group can be found in legend of Fig. 2. The dose of ACTH in this experiment was 0.1 μg per 3 μl in order to induce a grooming score comparable to dynorphin-induced grooming. The control animals received at T=0 saline, whereas the experimentals received the peptide. All animals received at T=4 the concerning peptide in study (see [14]). Two types of comparisons were made: experimentals were compared to controls at T=4 and the grooming score of the experimentals at T=0 was compared with their grooming score at T=4.

In a third experiment the blockade of dT-Dyn-induced grooming response by pretreatment with naloxone was investigated. Two groups (both 5 animals per group) were given dT-Dynorphin (1 μg/3 μl); one was pretreated with naloxone (1 mg/kg) and the other with saline.

Statistics

For comparing different groups, statistical analysis was performed by analysis of variance (F-test with a completely randomized design) followed by an appropriate supplemental *t*-test on the square root of the accumulated grooming scores or of the score per separate grooming element [4].

Since two grooming scores per individual were recorded in the cross-tolerance experiments a *t*-test for the comparison of experimentals versus controls was supplemented with a *t*-test for related measures of the comparison experimentals at T=0 versus experimentals at T=4. A Student's *t*-test was used for testing naloxone-pretreated against saline-pretreated animals in experiment three.

Peptides

Where indicated, naloxone (Endo, New York; 1 mg/kg, SC) or saline (0.2 ml) was given 5 min prior to ICV peptide treatment. The synthetic peptides ACTH(1–24), dynorphin(1–13) and des-tyrosine[1]-dynorphin(1–13) were obtained from Organon Int. B.V. (Oss, The Netherlands).

RESULTS

In confirmation of previous studies, we have found that

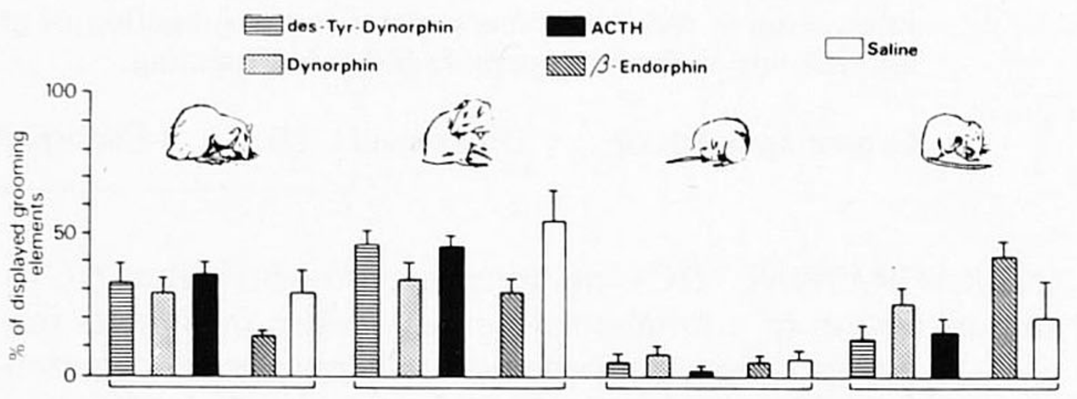

FIG. 3. The composition of the grooming induced by several peptides. As is drawn in top of the figure (from left to right respectively) head washing, bodily grooming, sexual grooming and scratching were distinguished. The proportion of each of the four grooming elements (relative to the total grooming score) was calculated for each peptide. ACTH(1–24) (1 μg), dynorphin (1 μg) and dT-Dyn (1 μg) show a similar grooming pattern which for the elements head washing and scratching is significantly different from that elicited by β-endorphin (0.3 μg).

ACTH(1–24), and dynorphin(1–13) induce excessive grooming behavior (Fig. 1) [8, 10, 23]. We now report that the non-opioid peptide, dT-Dyn also induces excessive grooming (Fig. 1). The grooming induced by this non-opioid fragment could be blocked by pretreatment with the specific opiate antagonist, naloxone (Table 1). As was demonstrated previously for ACTH(1–24) [14] and β-endorphin [22], it is now reported for dT-Dyn that a second administration of the peptide after short interval does not lead to an excessive grooming response (Fig. 2 A,F). As reported previously for ACTH(1–24) and β-endorphin, if at T=0 saline was given and at T=4 dT-Dyn, a normal dT-Dyn grooming response was observed. Conversely if at T=0 dT-Dyn was given and at T=4 saline, no excessive grooming was seen at T=4. Furthermore, the treatment group saline T=0 and saline T=4 did not show excessive grooming at both time points (data not shown, see also [14,21]). In addition, we studied the possible cross-tolerance between dT-Dyn and both dynorphin(1–13) and ACTH(1–24). As is shown in Fig. 2B and 2C for dT-Dyn and dynorphin(1–13) and in Fig. 2D and 2E for dT-Dyn and ACTH(1–24), a clear cross-tolerance for

the grooming response could be calculated. Per animal the second grooming score is presented in Fig. 2 as a percentage of the first. Both the *t*-test for related measures for comparing two treatments per animal and the normal *t*-test for comparing saline-treated with peptide-treated animals lead to significant results ($p<0.05$) in all the experiments.

As reported previously, the structure of grooming behavior after ICV ACTH(1–24) is similar to that observed in rats after novel or stressful procedures like the ICV injection of saline [11,16]. Figure 3 illustrates that ACTH(1–24), dynorphin and dT-Dyn show a similar grooming pattern, which is different from that seen after ICV β-endorphin. Statistical analysis revealed that rats treated with β-endorphin displayed less head washing and more scratching, respectively (F-value for head washing 4.975 $df1/df2=24/4$, 2 $p<1\%$; F-value for scratching 5.9 $df1/df2=24/4$, 2 $p<1\%$). Removal of the N-terminal Tyr-residue from dynorphin(1–13) does not influence the characteristic pattern of grooming elements displayed as there are no significant qualitative differences between dynorphin and its des-Tyr fragment.

DISCUSSION

We have investigated the importance of an interaction of a peptide with an opiate receptor for the induction of excessive grooming behavior. Certainly, the blockade of morphine-, β-endorphin-, dynorphin- and ACTH-induced grooming by specific opiate antagonists points to an opiate-sensitive system as part of the pathway leading to the expression of grooming behavior [11,12]. However, the question is whether direct interaction with an opiate receptor is required to initiate grooming behavior. We have taken advantage of the known structural requirements for peptide binding to the opiate receptor in order to answer this question. The removal of the N-terminal tyrosine from opiate peptides such as β-endorphin, enkephalin or dynorphin drastically reduces the affinity of these peptides for their respective receptors [5, 7, 19].

We have shown that the des-tyrosine fragment of dynorphin is still capable of inducing excessive grooming (Fig. 1). For this peptide it can be concluded that opiate receptor affinity is not required for the induction of this behavior. However, two apparently opiate-like features of the behavioral response are retained; acute tolerance develops and naloxone pretreatment blocks the peptide-induced grooming. Since different peptides—such as β-endorphin—induce different grooming patterns, the resemblance between dynorphin and dT-Dyn with respect to the composition of grooming elements is in agreement with an unchanged peptide/neural substrate interaction. As discussed elsewhere [11] the single-dose tolerance observed in peptide-induced grooming seems to reflect a property of the neural substrate.

TABLE 1

INHIBITION OF dT-DYN-INDUCED GROOMING BEHAVIOR BY NALOXONE

Treatment	N	Grooming score (mean ± S.E.M.)	
Saline-dT-Dyn	4	96 ± 5	$p<0.02$
Naloxone-dT-Dyn	5	19 ± 4	

Naloxone (1 mg/kg) or saline was administered SC, 5 min prior to ICV administration of peptide (dT-Dyn, 1 μg/3 μl). N, indicates the number of animals per treatment. The analysis was performed with a Student's *t*-test.

The cross-tolerance observed here implies that the pathways of the peptide-activated substrate for both the ACTH and dynorphin converge.

The data presented support the view that, at least for dynorphin-induced grooming the primary event is not activation of an opiate receptor. The fact that the behavior seen after the administration of the des-Tyr peptides can still be blocked by naloxone is a reflection of the fact that the neural substrate underlying peptide-induced grooming contains opiate receptors [11]. Analysis of the temporal aspects of ACTH and novelty/stress-related grooming suggests that this opiate receptor system is secondary in time to a non-opiate-sensitive system [12]. In addition, the substrate contains dopaminergic pathways as well [11, 12, 16, 20]. Hence it is not surprising that systemic treatment with haloperidol blocks dT-Dyn-induced excessive grooming [2]. It should be noted that, as previously reported for ACTH [20], dynorphin [21] and β-endorphin [21], the grooming induced by the des-Tyr peptide could be blocked by pretreatment with the specific dopamine antagonist haloperidol [2]. Data obtained with other peptides also point to a dissociation of affinity for opiate receptors from grooming induction. For example, α-MSH [Ac-ACTH(1–13)-NH_2] and ACTH(1–24), have similar potency in eliciting grooming, yet α-MSH is devoid of affinity for brain opiate receptors [8, 15, 18]. Hence, we suggest that the opiate receptors are involved at a site different from the primary receptor(s) for grooming-inducing peptides.

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