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Effects of Steroids on the Olfactory Function of the Dog

PATRICK I. EZEH,* LAWRENCE J. MYERS,*†[1] LYNN A. HANRAHAN,‡
ROBERT J. KEMPPAINEN* AND KEITH A. CUMMINS§

**Department of Physiology and Pharmacology, †Institute for Biological Detection Systems, ‡Department of Pathology, and §Animal and Dairy Science, Auburn University, Auburn, AL 36849*

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EZEH, P. I., L. J. MYERS, L. A. HANRAHAN, R. J. KEMPPAINEN AND K. A. CUMMINS. *Effects of steroids on the olfactory function of the dog.* PHYSIOL BEHAV **51**(6), 1183–1187, 1992.—Twenty-four (24) mature, mixed breed, healthy dogs weighing from 14.6 kg to 27.6 kg were used to study the effects of various steroids on the olfactory function of the dog using olfactory detection threshold as an index. Two odorants were used, viz; benzaldehyde and eugenol. Of the various steroids used, only dexamethasone produced classical signs of Cushing's syndrome in the dogs. However, all dogs that received either dexamethasone alone or hydrocortisone plus DOCA exhibited a significant elevation in the olfactory detection threshold for both odorants without any observable structural alteration of the olfactory tissue using light microscopy. On the other hand, neither DOCA, hydrocortisone alone, nor any of the vehicles used in the study significantly altered the olfactory function of the dogs. The results show that Cushing's syndrome can be experimentally produced in dogs using exogenous steroids and that this condition diminishes the olfactory capability of the dog without producing classical signs of the disease.

Steroids Dog olfaction Alteration Detection threshold

THE conditions known to alter olfactory function are varied in their origin. These can be nervous, nutritional, local, viral, or endocrine (8,10,13,16,19). While most of the data on olfactory alterations have been obtained from humans, canine olfactory abnormalities have been the source of many complaints by clients, especially owners of field-trial and hunting dogs (11,15).

The olfactory system has been shown to be altered by both deficiency and excess of steroids, especially glucocorticoids (7,9,19). The authors reported that in patients with adrenal insufficiency, there is a marked increase in the ability to detect odorants, while in patients with adrenal hypersecretion (Cushing's syndrome), there is a decrease or absence of the ability to detect odorants. Although there is general agreement on the above, there is individual variation with respect to olfactory capability (1,20). Most, if not all, the data available are from human subjects; thus, their olfactory function is evaluated while the condition has been in existence for some time, and the conclusion is based on comparison of such data with what the investigators considered "normal" values (3,5,6,8). Such conclusions must be viewed with caution in view of individual variation of olfactory function and the absence of data prior to existence of the condition. In addition, there are no studies available which contain both pre- and postcondition data or that provide information on whether (Cushing's) syndrome alters the structural integrity of the system.

The objectives of this study were to explore the possibility of producing Cushing's syndrome in dogs using various synthetic steroids; to investigate the effect(s) of such intervention on the olfactory function of the animals utilizing the olfactory detection threshold as an index or criterion; and to investigate the structural alteration of the olfactory system by the steroids.

The dog was chosen as a model based on the following: a) Cushing's syndrome is common in dogs. b) Producing the condition enables collection of pre- and postcondition data, accounting for individual variation. c) The olfactory sensitivity of the dog is higher than that of man, and as such, any alteration in olfactory function will likely be more dramatic in this species than in humans. d) There exists a simple, experimental procedure for the evaluation of the olfactory function in dogs e) The opportunity exists for the evaluation of the structural characteristics of the olfactory system under such conditions.

METHOD

Animals

Twenty-four (24) mature, mixed breed, healthy dogs (11 males and 13 females), weighing from 14.6 kg to 27.6 kg (32 to 61 lb), were used in this study. The dogs were housed in individual cages with free access to food and water throughout the study. In addition, the health status of the dogs was closely monitored during the same period, and the clinical status recorded.

Adrenal Function Test

The functional status of the adrenal glands was tested utilizing the ACTH stimulation test (12). This was carried out by the

[1] Requests for reprints should be addressed to Lawrence J. Myers, 217 Greene Hall, Auburn University, Auburn, AL 36849.

intravenous injection of synthetic ACTH (Cortosyn; Organon Inc., West Orange, NJ) at a dosage level of 0.25 mg per dog. Blood samples were collected before and 1 hour after the injection of ACTH using heparinized vacutainer glass tubes (Becton Dickinson and Company, Rutherford, NJ), for the determination of the plasma cortisol levels. The plasma cortisol concentrations were determined using a commercial radioimmunoassay (RIA) kit (Coat-A-Count; Diagnostic Products, Los Angeles, CA). The pre- and post-ACTH cortisol levels were analyzed and only dogs showing concentrations within the normal range (10.0 to 90.0 and 220 to 560 nmol/L, respectively) were used for the study. The dogs used in this study had their mean pre- and post-ACTH cortisol levels as 49.7 ± 19.3 and 344.9 + 38.6 nmol/L (mean ± SEM), respectively.

Odorants Preparation

Benzaldehyde and eugenol (Sigma Chemical Company, St. Louis, MO) samples were serially diluted in propylene glycol (Fisher Scientific Company, Fair Lawn, NJ) in 16 ten-fold increments and were identified as a negative log of dilution from 0 to 15, with 0 as the most concentrated sample, and 15 the least concentrated sample. The concentration of the benzaldehyde sample 0 was 9.89 M, while the concentration of eugenol sample 0 was 6.52 M. Each dilution was separately stored in a rack, and care was taken to avoid cross-contamination. Benzaldehyde and eugenol were selected because benzaldehyde is known as a trigeminal receptor stimulant as well as an olfactory nerve stimulant; eugenol is known as a relatively pure olfactory receptor stimulant (6,7).

Dog Preparation

Dogs were prepared according to earlier described techniques, behavioral and electroencephalographic olfactometry (21). These techniques were performed in a well-ventilated and temperature-controlled (25°C + 1°C) room. White noise was provided to prevent interference by external auditory stimuli. Dogs were placed in right lateral recumbency without chemical restraint. Lidocaine hydrochloride (0.4%) (LymphoMed, Inc. Rosemont, IL) was injected into standard sites on the scalp for electroencephalographic (EEG) recording, and clamp electrodes were placed accordingly (25). Two platinum subdermal, type E2 electrodes (Grass Instrument Company, Quincy, MA), were placed in the splenius muscle near the dorsal midline of the neck to record the electromyographic (EMG) activity. In addition, electrodes were placed subcutaneously at the dorsum of the thorax and another at the ventrum of the thorax to record the electrocardiogram (ECG). An EEG model 8-10D (Grass Instrument Company, Quincy, MA) was used to record the activity. The dog was then blindfolded. Dogs were calm prior to odor presentation, as indicated by minimal spontaneous movement on the part of the dog.

Determination of the Olfactory Detection Threshold

The olfactory function of the dogs was evaluated by determining the olfactory detection threshold of each dog for benzaldehyde and eugenol according to a previously described technique (15). This was done before and throughout the medication period, and were never performed within an hour of the time of injection of a dog. The premedication detection threshold was determined in triplicate (each determination done on a separate day) for each dog and for each odorant, and the mean value was taken as the normal olfactory detection threshold of that dog for the specific odorant. Dogs that had a mean detection threshold within the established normal range for both odorants were used for the study, while those that did not meet the above requirement were rejected.

The odorant solutions were presented to the dogs in ascending order of concentration starting with a glass holder used to present the solutions. This was followed by the presentation of propylene glycol, then the most dilute sample, and proceeding with the next more concentrated sample, and so on in that order. Each sample was opened and placed approximately 2 cm ventral to the tip of the dog's nose. Each dilution was held in place for 10 seconds, withdrawn for 15 seconds, and then followed by the presentation of the next sample.

Response to presentation of olfactory stimuli was evaluated by visual examination and manual analysis of the EEG and EMG by observation of the dog's behavioral response. The olfactory detection threshold of each dog for a specific odorant is the lowest concentration of that odorant which elicited an electroencephalographic and/or electromyographic alerting response from the animal and/or specific behavioral response from the animal. The EEG alerting response is characterized as a stimulus-associated reduction of the amplitude and increase in frequency of the EEG waveforms (18). The specific behavioral responses utilized were sniffing and head movements spatially directed to the odorant sample presentation.

Steroid Administration

The dogs were randomly divided into six groups, with four dogs per group. Each group was treated with the appropriate steroid(s) or the vehicle (normal saline or peanut oil) (Table 1). The dogs were treated once daily for 28 consecutive days using the intramuscular route of administration.

Olfactory Tissue Samples Collection

At the end of the treatment period, the dogs were euthanized by intravenous injection of a commercial euthanasia solution (T-61 Euthanasia Solution, National Laboratories Corp.) and the ethmoid concha collected immediately from both sides of the nasal cavity. A portion of this tissue was flash frozen in liquid nitrogen and immediately stored at −70°C for later enzyme analysis, while a portion was fixed in buffered 10% formalin for histological evaluation.

Histological Evaluation

The fixed tissues were sectioned at 5 microns, stained with hematoxylin and eosin (Mayer's method), and evaluated using light microscopy.

TABLE 1
SCHEDULE OF TREATMENT

Group	Drug	Dosage
1	Dexamethasone*	2.0 mg/kg
2	Hydrocortisone† and DOCA‡	0.25 mg/kg
3	Hydrocortisone only	0.25 mg/kg
4	DOCA only	0.25 ml/kg
5	Peanut oil¶	0.25 ml/kg
6	Normal saline	0.25 mg/kg

Each dog was treated accordingly for 28 consecutive days.
* Burns Veterinary Supply, Oakland, CA.
† Schein Pharmaceutical Inc., Port Washington, NY.
‡ Sigma Chemical Company, St. Louis MO.
¶ Used as the vehicle for DOCA.

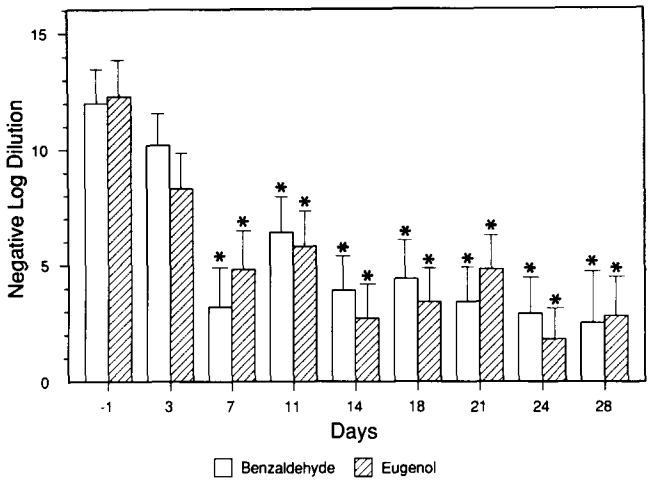

FIG. 1. Olfactory detection threshold for the dexamethasone-treated dogs (group 1) (mean + SEM). *Significantly higher than the pretreatment threshold (days: −1 = pretreatment period; 3 through 28 posttreatment period).

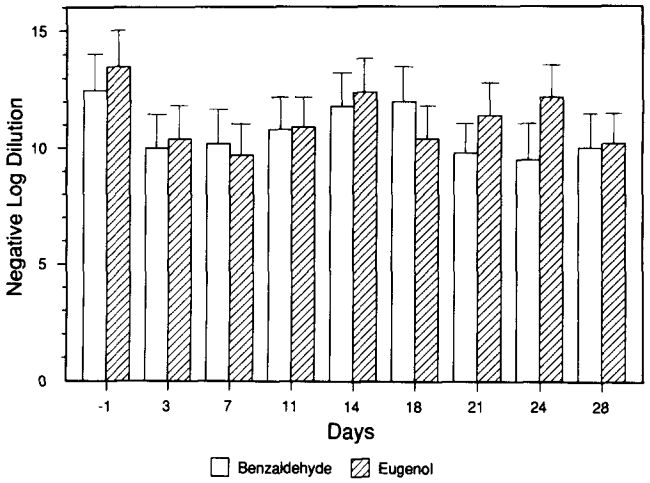

FIG. 3. Olfactory detection threshold for the HC-treated dogs (group 3) (mean + SEM) (days: −1 = pretreatment period; 3 through 28 post-treatment period).

Statistical Analysis

Statistical analysis was performed using the PROC GLM procedure of the SAS system for the analysis of covariance. The covariant was the mean pretreatment detection threshold for each dog. The model included group, time posttreatment, the interaction of group and time posttreatment, and the covariant as independent variables.

RESULTS

Clinical Observations

All dogs in the dexamethasone-treated group (Group 1) exhibited clinical signs characteristic of Cushing's syndrome. These included polydipsia, polyuria, and polyphagia. Other signs observed were pendulous abdomen, muscle weakness, and increased panting. No attempt was made to assign a clinical score to the severity of the signs.

On the other hand, none of the dogs that received either HC + DOCA, HC only, DOCA only, peanut oil or normal saline (groups 2, 3, 4, 5, and 6, respectively) exhibited any of the classical signs of Cushing's syndrome. They all appeared to be healthy throughout the study period.

Olfactory Detection Threshold

The results showed significant alterations in the detection threshold for both benzaldehyde and eugenol in the dogs that received either dexamethasone or HC + DOCA, i.e., groups 1 and 2, respectively, though the time of onset of the alterations varied between the two groups. The alterations were in the form of elevations in the olfactory detection threshold reflecting decreased olfactory acuity (Figs. 1 and 2).

The dogs in groups 3 and 4 (HC- and DOCA-treated dogs, respectively) did not show significant alterations in their detection

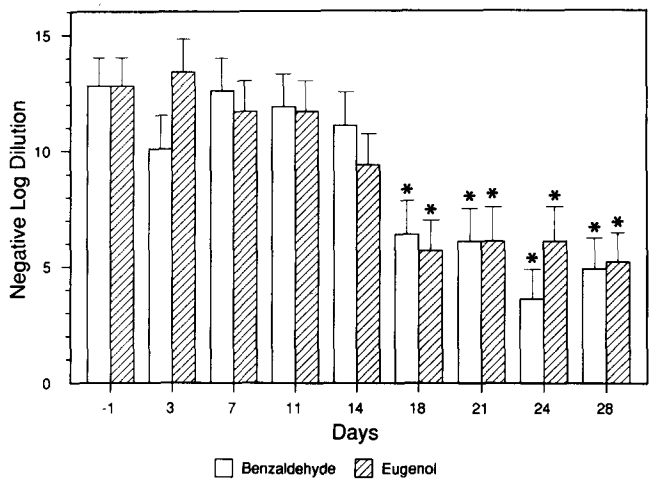

FIG. 2. Olfactory detection threshold for the DOCA- + HC-treated dogs (group 2) (mean + SEM). *Significantly higher than the pretreatment threshold (days: −1 = pretreatment period; 3 through 28 posttreatment period).

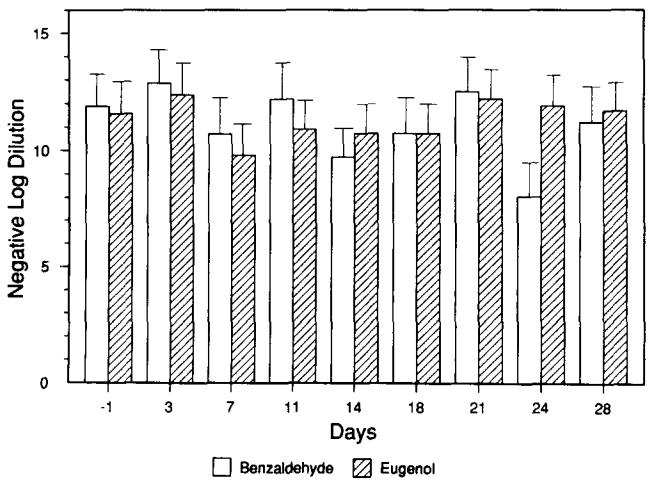

FIG. 4. Olfactory detection threshold for the DOCA-treated dogs (group 4) (mean + SEM) (days: −1 = pretreatment period; 3 through 28 post-treatment period).

thresholds (Figs. 3 and 4). On the other hand, the dogs in groups 5 and 6 exhibited a consistent detection thresholds throughout the study period (Figs. 5 and 6) when compared to the pretreatment threshold for the respective group.

Steroids and Olfaction in the Dog

In all the groups and throughout the study period there was no significant difference between the detection thresholds for the two odorants within a group and at a specific time during the study. The data also show that there was no significant difference in detection threshold among the groups prior to the initiation of the treatments. This was not the case during part of the posttreatment period.

Dogs in group 1 showed a significant ($p < 0.002$) elevation in olfactory detection thresholds within the first week, i.e., 7 days of medication (Fig. 1). While the pretreatment detection thresholds were 12.0 + 1.4 and 12.3 + 1.5 (mean ± SEM) for benzaldehyde and eugenol, respectively, 7 days postmedication thresholds were 3.2 + 1.7 and 4.8 + 1.5, respectively, values that were significantly ($p < 0.002$) higher than the pretreatment values. This trend in significant olfactory detection threshold elevation continued throughout the study period for dogs in this group.

Group 2 dogs, on the other hand, did not exhibit significant elevations in detection threshold during the first 2 weeks of medication (Fig. 2). However, the detection thresholds were significantly elevated during the last 2 weeks of medication in this group. At day 18 posttreatment, the detection thresholds were 6.4 + 1.4 and 5.7 + 1.3 for benzaldehyde and eugenol, respectively; values that were significantly ($p < 0.03$) higher than the corresponding pretreatment detection thresholds (12.8 + 1.2 and 12.8 + 1.2, respectively). As in group 1 dogs, this trend in detection threshold elevation continued throughout the last 2 weeks of medication. It should be mentioned that some of the dogs in groups 1 and 2 did not respond to even the most concentrated dilution of the odorants at some of the posttreatment time periods. These were not included in the statistical analysis and, as such, the elevation in the detection thresholds presented here are probably underestimated.

Histological Findings

There were no apparent morphological alterations to the olfactory epithelium of any dog in any group, either by gross or histologic examination. One dog in group 4, however, was found to have acute eosinophilic rhinitis. This was suggestive of allergic rhinitis and it extended to all tissues of the nasal cavity (both olfactory and respiratory areas), and was considered unrelated to the treatment. This condition may have been responsible for the slight alteration in olfactory detection threshold observed in this dog. As a result, data from the dog were not included in the mean detection threshold data presented here.

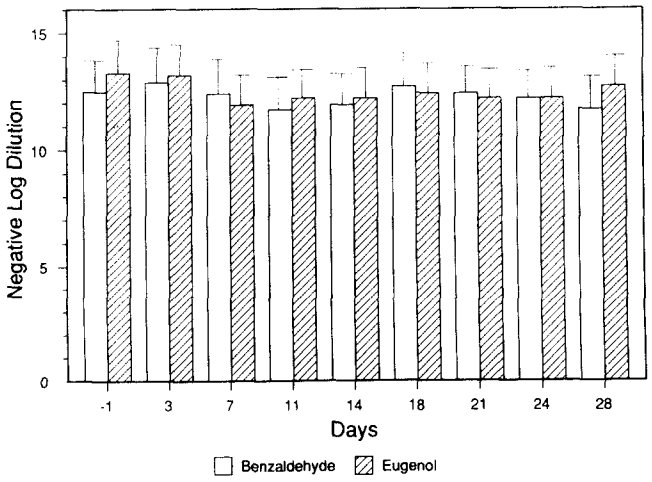

FIG. 5. Olfactory detection threshold for the peanut oil-treated dogs (group 5) (mean + SEM) (days: −1 = pretreatment period; 3 through 28 posttreatment period).

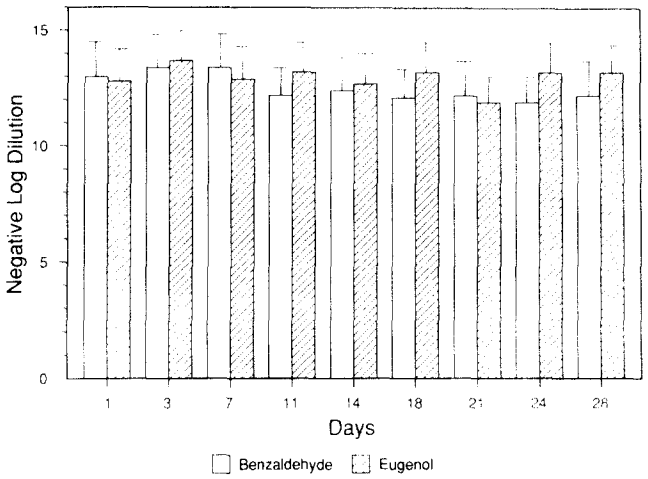

FIG. 6. Olfactory detection threshold for the normal saline-treated dogs (group 6) (mean + SEM). (Days: −1 = pretreatment period; 3 through 28 posttreatment period).

DISCUSSION

The most significant finding of this study was that dogs receiving either dexamethasone or hydrocortisone and DOCA showed an increase in the olfactory detection threshold for benzaldehyde and eugenol. The time of onset of the significant elevation in detection threshold in the two groups of dogs differed (Figs. 1 and 2). These results are logically consistent with previous findings in which the olfactory system has been shown to be affected by an excess of steroids, especially glucocorticoids (7,9,19). The condition increases the olfactory detection threshold for various odorants. Dexamethasone did this when used alone at the dosage level utilized, but both DOCA and hydrocortisone when used alone failed to do so. The finding that DOCA and hydrocortisone produced an elevation of threshold when used in combination suggests that there is either an additive or synergistic effect.

On the other hand, in those dogs that received either hydrocortisone, DOCA, normal saline, or peanut oil there was no significant alteration in the detection threshold for either benzaldehyde or eugenol (Figs. 3–6).

This study also showed that while dexamethasone-treated dogs showed classical signs of Cushing's syndrome, those that received DOCA and hydrocortisone did not. The finding, however, shows that the olfactory alteration could occur without observable clinical signs. Thus, while other conditions may also lead to elevation in olfactory detection threshold (19), it may be advisable that observations of threshold alteration be followed by adrenal function test where appropriate (12) to help in early detection of the disease. These variations in the two groups may be due to either the steroid and/or dosage level used. It should be pointed out, however, that during the period of olfactory alteration in both groups 1 and 2 dogs, there was no significant

difference in the degree of alteration. This shows that the type of steroid and/or dosage may be important with respect to the time of onset only. Further studies are needed to clarify this question. Prolonged exposure to various steroids may eventually lead to olfactory alteration.

The finding that the alteration in detection threshold occurred with both odorants to the same degree suggests that both the olfactory and trigeminal components of the system might be involved, since eugenol is considered a pure olfactory stimulant and benzaldehyde is both an olfactory and trigeminal stimulant (3,4).

Possible approaches to follow in an attempt to determine the mechanism of the olfactory alteration include the determination of the occurrence of local atrophy, disruption or injury to the system, damage to the neural projections, disturbance of, or interference with, the neuronal regeneration, disruption of the receptor cells, modification of the fluids bathing the olfactory mucosa, or interference or alteration of various enzyme activities (19).

The observation that there was no structural alteration of the ethmoid concha in any dog in any group does not absolutely preclude any structural involvement in the alterations observed. It is possible that structural alteration may be detectable by other methods. Further studies are needed in this area.

The findings indicate that olfactory evaluation may be useful as an indication for additional diagnostic tests for Cushing's syndrome. This is supported by the elevation in olfactory detection threshold detected in subjects without observable clinical signs of the disease.

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