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TAXON-SPECIFIC DIFFERENCES IN RESPONSIVENESS TO CAPSACIN AND SEVERAL ANALOGUES: CORRELATES BETWEEN CHEMICAL STRUCTURE AND BEHAVIORAL AVERSIVENESS

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Abstract—The present set of experiments was designed to explore avian insensitivity to capsaicin. Based upon a molecular model of avian chemosensory repellency, we hypothesized that structural modifications of the basic capsaicin molecule, which is itself not aversive to birds, might produce aversive analogues. To this end, European starlings (Sturnus vulgaris) and Norway rats (Rattus norvegicus) were given varied concentrations of synthetic capsaicin and four analogues (methyl capsaicin, veratryl amine, veratryl acetamide, vanillyl acetamide) in feeding and drinking tests. The results agreed with a model that we are developing to describe the chemical nature of avian repellents. Synthetic capsaicin and vanillyl acetamide were not repellent to birds, owing to the presence of an acidic phenolic OH group. Conversely, veratryl acetamide was aversive, due to the basic nature of this compound. For rats, repellent effectiveness among compounds was reversed: synthetic capsaicin was the best repellent while veratryl acetamide was the worst. We speculate that this taxonomic reversal may reflect basic differences in trige-

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minal chemoreception. In any case, it is clear that chemical correlates of mammalian repellents are opposite to those that predict avian repellency.

Key Words—Capsaicin, chemosensory, irritation, rat, Rattus norvegicus, starling, Sturnus vulgaris, trigeminal.

INTRODUCTION

Avoidance of a chemical can be learned by associating sensory features of the chemical with post-ingestional effects, e.g., sickness (Riley and Clarke, 1977). Alternatively, avoidance of a chemical can occur in the absence of learning, provided that the chemical stimulates trigeminal free nerve endings (Parker, 1912) in the nose, eyes, or mouth. While the morphological organization of the trigeminal system in birds is not very different from that found in mammals (Dubbeldam and Veenman, 1978), there appear to be dramatic behavioral discrepancies (Kare and Mason, 1986). Although the avian trigeminal system responds to odorants (e.g., Walker et al., 1979, Mason and Silver, 1983) and mediates avoidance of avian irritants, e.g., methyl anthranilate (Mason et al., 1989), it does not respond to strong mammalian irritants. Thus, pigeons (Columba livia) and grey partridges (Perdix perdix) are indifferent to ammonia (Soudek, 1929), and parrots (Amazona spp., Mason and Reidinger, 1983), pigeons (Szolcsanyi et al., 1986), red-winged blackbirds (Agelaius phoeniceus; Mason and Maruniak, 1983), European starlings (Mason and Clark, 1990), cedar waxwings (Bombycilla cedrorum), and house finches (Carpodacus mexicanus; D. Norman, unpublished observation), are insensitive to capsaicin, the pungent principle in Capsicum peppers.

The finding that birds are unresponsive to capsinum is especially interesting, because mammals uniformly avoid this substance. The present set of experiments was designed to explore this instance of avian insensitivity. On the basis of a model relating avian repellency with chemical structure (Mason et al., 1991; Clark and Shah, 1991; Clark et al., 1991; Shah et al., 1991), we hypothesized that structural modifications of the basic capsaicin molecule might produce analogues that were aversive to birds. To this end, starlings and Norway rats (Rattus norvegicus, laboratory strain) were given varied concentrations of capsaicin and four analogues (methyl capsaicin, veratryl amine, veratryl acetamide, vanillyl acetamide) in feeding and drinking tests.

Our choice of analogue structures was guided by the following considerations. First, methyl capsaicin was selected because its additional OCH₃ group (Figure 1) made it more basic than capsaicin, and molecular basicity is positively associated with avian repellency (Clark and Shah, 1991). Second, vanillyl acetamide was chosen because the removal of the alkyl group (relative to capsaicin) should enhance repellency by contributing to the electron richness of
Fig. 1. Molecular structure of synthetic capsaicin, methyl capsaicin, veratryl amine, vanillyl acetamide, and veratryl acetamide.

the phenyl ring (Shah et al., 1991). Third, veratryl amine was evaluated because it is more basic than capsaicin and because there is no possibility of side chain interference in effectiveness, e.g., no possibility of electronic interference (Clark et al., 1991). Finally, we selected veratryl acetamide both because it is basic relative to capsaicin or vanillyl acetamide, and because it is more lipophilic than veratryl amine. Because lipophilicity is positively correlated with repellency (Mason et al., 1989), we predicted that veratryl acetamide should be the most effective bird repellent of the derivatives tested.

MATERIALS AND METHODS

Subjects. Adult European starlings (Sturnus vulgaris) were decoy-trapped in Pilesgrove township, Salem County, New Jersey, and transported to the laboratory. Birds were held for 3 weeks prior to testing. We chose to use this
species because (1) they show good chemical sensing ability (e.g., Clark and Mason, 1987), and (2) comparable data exist concerning the responses of starlings to other repellents, e.g., anthranilate derivatives (Mason et al., 1989) and mammalian irritants, e.g., piperine, zingerone, allyl isothiocyanate, and gingerol (Mason and Otis, 1990).

Upon arrival, the birds were individually caged (61 x 36 x 41 cm) under a 12:12 light-dark cycle with light onset at 0700 hr. Food and grit [Purina Flight Bird Conditioner (PFBC) and medicated oyster shells] were available ad libitum; apples were provided twice a week. Before experiments began, birds were permitted free access to tap water.

Sixty-day-old Sprague-Dawley Norway rats were obtained from the Vassar College colony. The rats were individually caged (17.7 x 24.2 x 17.7 cm) under a 12:12 light/dark cycle (light onset 0700 hours). During a 2-week adaptation period prior to testing, all rats were permitted free access to crumbled Purina rat chow and tap water.

**Chemicals.** Veratryl amine was purchased from Fluka Chemical Co. (Buchs, Switzerland), while synthetic capsaicin was obtained from Aldrich Chemical Co. (Milwaukee, Wisconsin). Vanillyl acetamide was synthesized according to the method described by Helson (1919). Methyl capsaicin (corresponding to synthetic capsaicin, i.e., veratryl nonamide) and veratryl acetamide were synthesized by a simple acetylation procedure, as follows. Veratryl amine (10 g) was dissolved in triethylamine (20 ml) and the solution was cooled to 0°C. The nonanoyl chloride or acetic anhydride (two equivalents) was added dropwise and the solution was allowed to stand at room temperature for 1 hr. The reaction mixture was poured into ice water (50 ml) and extracted with dichloromethane (2 x 25 ml). The organic layer was successively washed with 5% HCl (25 ml), 11% sodium hydrogen carbonate (25 ml), and water (25 ml), dried and evaporated under reduced pressure. The residue was purified by column chromatography (on silica gel) to obtain pure samples of veratryl nonamide and veratryl acetamide.

For the bird experiments, each chemical constituted an independent test for both the feeding and drinking experiments. For each chemical in drinking trials, stimuli were dissolved in water to prepare the following concentrations (w/v): 0.001, 0.005, 0.01, 0.05, 0.1, and 1.0%. For each chemical in feeding trials, stimuli were first dissolved in ether, and the solutions applied to bird feed. The ether was then evaporated (Jakubas et al., 1991). Chemical concentrations used in the bird feeding trials were: 0.001, 0.01, 0.1, and 1.0% (w/w).

**Procedures.** In drinking trials, one-bottle tests similar to those described by Clark and Shah (1991) were used. Briefly, water consumption for starlings was monitored for a total of 6 hr for each of 3 days. At the end of this period, individuals whose variance was > ± 1 standard deviation of the population vari-
ance were excluded from the trials. Those birds with stable daily water consumption were ranked according to mean water consumption and assigned to one of the six treatment concentration groups (hereafter called concentration group). That bird with the highest water consumption was assigned to the 0.5% concentration group, that bird with the second highest consumption, to the 0.1% concentration group, and so forth, until all birds were assigned to a group. This assured that all concentration groups were balanced with respect to drinking when trials began. A total of 36 birds was used for each experiment, with six birds per concentration group. During any given experiment, only a single chemical was tested.

After assignment to a concentration group, a 1-day pre-treatment drinking trial began. At 0930, tap water consumption for birds within each concentration group was measured every 2 hr for a total of 6 hr. These measures served as the control level of water consumption. After the pre-treatment trial, drinking tubes were switched (to maintain consistent protocol) and the birds were provided free access to fresh tap water. The following day, at 0930, tap water was replaced with a preassigned concentration of chemical in water, and consumption was recorded every 2 hr for the next 6 hr. After the test, birds were again provided free access to tap water. Consumption of tap water was monitored overnight. Overnight consumption was monitored to evaluate whether birds made up for any water deficits resulting from experiments. During the post-treatment trial, tap water consumption was monitored every 2 hr for a total of 6 hr. Mean posttreatment water consumption within concentration groups was compared with mean pre-treatment water consumption to determine whether consumption had returned to pre-treatment levels; i.e., were there carryover effects due to consumption of treated water? If pre-treatment consumption was equal to posttreatment consumption, then the next compound was tested following the above procedures. During subsequent experiments with other chemicals, each group was assigned a different concentration, as determined by a counterbalanced predetermined schedule. This was done to minimize the possibility that strength of stimulus, rather than quality per se affected drinking behavior. Previous experience suggested that these precautions were adequate, though we did not specifically test for this effect.

Protocol specified that an individual's intertrial water consumption be maintained within \pm 1 standard error of its pre-treatment value. If this were the case, the bird was used in the next experiment; otherwise, it was replaced with a new bird whose baseline water consumption was matched for the group mean. Individual birds showed remarkable consistency for water consumption. Only one bird was replaced throughout the drinking trials.

Birds were checked for health condition after each daily trial, e.g., unusual inactivity, vent fouling, and piloerection. There were no mortalities, and birds were released to the wild at the end of the experiment. Naive birds were not
used for each test because it was impractical to capture the 180 starlings required for all trials.

In feeding trials, 24 starlings were used with each chemical. In every test, the birds were given a 4-day pre-treatment period, during which each was presented with a cup containing 50 g of feed during the first 2 hr following light onset. Consumption was then assessed and birds were left with undisturbed access to the maintenance diet until lights were out. Feed was removed from the cages overnight so that the birds were moderately food deprived. On the fourth day, birds were assigned to four concentration groups (n = 6/group) on the basis of consumption. That bird with the greatest consumption was assigned to the first group, that with the second highest was assigned to the second group, and so forth.

A 4-day treatment period immediately followed pre-treatment. During the first 2 hr following light onset, each bird was given 1 cup containing 50 g of feed. The feed was adulterated with one of the stimulus chemicals, and different groups were presented with different concentrations. As in pre-treatment, consumption was measured after 2 hr, and birds were food-deprived overnight.

Rat feeding trials were similar to starling feeding trials, with two differences. First, rats were given only one concentration of each chemical (1.0%, w/w). We had planned to use the same range of concentrations presented to birds, but only capsaicin was aversive in pilot testing at a concentration of 0.1%. Second, a control group that received untreated chow during the treatment period was included. Drinking trials were not conducted because 1.0% concentrations of the chemicals were near the limit of solubility in the drinking trials with birds.

Analyses. For starling drinking trials, data were transformed using a difference score to control for individuals’ pre-treatment water consumption, i.e., treatment–pre-treatment, and posttreatment–pre-treatment. Difference scores were evaluated in a two-factor analysis of variance (ANOVA), with repeated measures between difference scores (two levels). The independent factor was treatment concentration group. Missing data was only a problem for the drinking experiments. At times, individuals would rest atop the drinking tubes and spill water. If this occurred during any of the test periods (pre-treatment, treatment, or posttreatment), that individual’s consumption was not included in the analysis.

For starling feeding trials, mean consumption was evaluated using three-factor ANOVAs with repeated measures between periods (two levels: pre-treatment, treatment) and among days (four levels). The independent factor was concentration group (four levels).

For rat feeding trials, mean consumption during the treatment period was assessed in a two-factor ANOVA with repeated measures among days. The independent factor was chemicals (six levels: the five chemicals, and a control).
In all cases, Tukey \textit{b post-hoc} tests were used to isolate significant differences among means \((P < 0.05)\). Unless otherwise indicated, all data were tested and found to be homogeneous using Bartlett's-Box method.

RESULTS

\textit{Avian Drinking Trials}

\textit{Synthetic Capsaicin}. There were no significant concentration, treatment period (block), or interaction effects (Figure 2A).

\textit{Methyl Capsaicin}. The interaction between concentration groups and period was significant \((F = 5.3; 5.29 \text{ df; } P < 0.001)\). \textit{Post-hoc} tests showed that, relative to posttreatment, there was a significant, albeit slight reduction in treatment difference scores for the highest methyl capsaicin concentration group (Figure 2B).

\textit{Vanillyl Acetamide}. The interaction between concentration groups and period was significant \((F = 6.3; 5.29 \text{ df; } P < 0.001)\). \textit{Post-hoc} tests showed that the mean treatment difference score for the highest concentration group was significantly less than the mean posttreatment score for that concentration group (Figure 2C).

\textit{Veratryl Amine}. The interaction between concentration groups and period was significant \((F = 5.5; 5.29 \text{ df; } P < 0.001)\). \textit{Post-hoc} tests showed that the treatment difference scores for the two highest concentrations of veratryl amine were smaller than the posttreatment difference scores for these concentrations. The highest concentration produced the greater effect (Figure 2D).

\textit{Veratryl Acetamide}. The interaction between concentration groups and period was significant \((F = 14.4; 5.29 \text{ df; } P < 0.001)\). \textit{Post-hoc} tests showed that while posttreatment difference scores remained constant or became larger, treatment scores decreased with increasing concentration. The two highest concentrations produced a drop from pre-treatment consumption that was significantly greater than the drop produced by any other concentration (Figure 2E).

\textit{Avian Feeding Trials}

\textit{Synthetic Capsaicin}. There were no significant differences for concentration, treatment period, day, or the interaction effects (Figure 3A).

\textit{Methyl Capsaicin}. The day by period by concentration interaction was significant \((F = 3.0, 9.60 \text{ df; } P < 0.005)\). \textit{Post-hoc} tests showed that the profile for consumption for the first day of pre-treatment differed from all other profiles. Indeed, all interaction terms and main effects involving days pointed to decreased consumption on the first day. However, more importantly to the hypothesis of interest, there was no period effect \((P = 0.789)\), even though the
Fig. 2. Mean consumption of synthetic capsaicin and the four capsaicin analogues in avian drinking trials. Open circles represent treatment-pre-treatment values, while filled circles represent mean posttreatment-pre-treatment values. Capped vertical bars represent standard errors of the means.

period by concentration profiles differed ($F = 5.4$, 3,20 df, $P < 0.007$; Figure 3B).

*Vanillyl Acetamide.* Again, there were no significant effects for treatment ($P = 0.51$), concentration ($P = 0.23$), or their interaction ($P = 0.69$; Figure 3C).

*Veratryl Amine.* Overall, the consumption of food was less during the treatment period relative to the pre-treatment period ($F = 635.4$; 1,20 df; $P < 0.001$). Inspection of the concentration by period interaction (Figure 3D) showed
that this relationship held down to the limits of concentrations tested, i.e., 0.001% (F = 44.62; 1,20 df; P < 0.001). Post-hoc tests showed that consumptions for birds assigned to the 0.1% concentration group did not differ between treatment periods.

Veratryl Acetamide. This compound did not appear to be as effective as veratryl amine, but was more effective than capsaicin, methyl capsaicin, and vanillyl acetamide. Overall, the treatment period consumption was less than the pre-treatment control (F = 67.15; 1,20 df; P < 0.001). The interaction of
period by concentration profiles indicated that the reduction in consumption of treated food was accentuated at higher concentrations (Figure 3E; $F = 33.63; 3,220 \ df; P < 0.001$). Post-hoc tests indicated that only the 1% concentration differed from pre-treatment levels and the lower levels of treated food.

Rodent Feeding Trials

Synthetic Capsaicin, Methyl Capsaicin, Veratryl Amine, Veratryl Acetamide, and Vanillyl Acetamide. There were significant differences among compounds ($F = 2.8; 5.18 \ df; P < 0.05$). Post-hoc examination of this effect indicated that synthetic capsaicin significantly reduced consumption relative to consumption by the group that received untreated feed. The least effective repellent was vanillyl acetamide; consumption by rats given this substance was significantly higher than the consumption exhibited by control group rats (Figure 4).

DISCUSSION

Although the peripheral trigeminal system in birds and mammals is morphologically similar, there are broad functional differences between the taxa. Birds are uniformly unresponsive to most (if not all) mammalian sensory irri-

![Diagram](image)

**Fig. 4.** Mean consumption in rat feeding trials with synthetic capsaicin and the four capsaicin analogues. All chemicals were presented at a concentration of 1.0% (w/w). Capped vertical bars represent standard errors of the means. The horizontal lines depict homogeneous group means as determined by a post-hoc Tukey's b test. Abbreviations: C = synthetic capsaicin, MC = methyl capsaicin, VRAM = veratryl amine, VRAC = veratryl acetamide, VNAC = vanillyl acetamide, CON = control.
tants at biologically relevant concentrations. The present set of experiments was designed to explore avian insensitivity.

Overall, the present results are consistent with our model describing the chemical nature of avian repellents (Clark et al., 1991; Clark and Shah, 1991; Mason et al., 1991; Shah et al., 1991). According to that model, the electron richness of the phenyl ring and basicity are positively related to repellency, whereas acidic functionalities are negatively associated with effectiveness. Hence, vanillyl amides, i.e., synthetic capsaiacin and vanillyl acetamide, should be weakly repellent (if at all), owing to the presence of the acidic phenolic OH group. Conversely, the veratralamide should be aversive.

Veratral amine and veratral acetamide were aversive to birds in both drinking and feeding trials. The former substance was the most basic of the compounds tested. These data agree with our earlier work on vanillyl and cinnamyl alcohol derivatives. In those experiments (Shah et al., 1991; Jakubas et al., 1991, respectively), veratral alcohol was more active than vanillyl alcohol and dimethoxy cinnamyl alcohol and its benzoate were more active than the corresponding coniferyl derivatives.

Methyl capsaiacin was not significantly repellent to birds in the present experiments, although the data hint that it may be aversive, relative to capsaiacin, but only at very high concentrations. The higher activity of veratral acetamide compared to veratral nonamide (methyl capsaiacin) may be due to stereoelectronic factors. For example, the nine-carbon chain on the amino group may sterically hinder the molecule’s activity, e.g., structural and conformational effects. Alternatively, this long chain could effectively block electronic effects of the electron-rich phenyl ring. However, steric effects alone are not sufficient to explain the inhibition of repellency. Indeed, the feeding experiments indicate that vanillyl acetamide was ineffective as a repellent. The fact that vanillyl acetamide was aversive at the highest concentration in drinking trials may reflect the tendency for drinking trials to be more sensitive (Clark et al., 1991), possibly because the active ingredients in solution have greater access to receptors than those attached to particles of food.

The interaction of steric and basic effects may be important for repellency. It is worthwhile to note that, structurally, both veratral amine and veratral acetamide have short (two-carbon) or no side chains. At present, the nature of the assay (feeding vs. drinking) precludes solution of which of these points is more important. Molecular modeling may clarify the importance of steric and electronic effects of the side chain on the basic molecule, as well as the importance of hydrophobic and hydrophilic interactions.

In mammals, repellent effectiveness among compounds was essentially reversed. Capsaicin was the best repellent while veratral acetamide was the worst. These results are consistent with the available evidence. We speculate that this taxonomic reversal may reflect several factors, e.g., differences in tri-
geminal chemoreception. In any case, it is clear that chemical correlates of mammalian repellents are opposite to those that predict avian repellency. Thus, acidity, a long side chain (lipophilicity), and an electron-poor phenyl ring may correlate positively with mammalian irritancy. According to the results obtained in this study and also the available data on the mammalian repellency of capsaicin derivatives (e.g., Green et al., 1990), the former two factors can be substantiated. More experiments are needed to test the third attribute.

LITERATURE


