COMPLEXITY OF CHEMICAL COMMUNICATION IN MAMMALS: URINARY COMPONENTS MEDIATING SEX DISCRIMINATION BY MALE GUINEA PIGS

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SUMMARY

Male guinea pigs are more attracted to urine from female guinea pigs than to urine from males. Separation of urine into fractions characterized by molecular weight and functionality through several different methods established that this discrimination and preference is based on a pattern of components which have widely different chemical character. The active compounds range from macromolecules (MW >10,000) to small, relatively volatile molecules. These studies establish a complexity in chemical signals that has not been previously documented for any other species.

INTRODUCTION

Intraspecies communication (1) through chemical signals is an important factor in the life cycle of many diverse species (2-5). These signals are mediated primarily through olfactory receptors and serve either by themselves or in conjunction with auditory, visual, or tactile signals to transmit information concerning species, sex, social status, and physiological and developmental states (2-5). Such phenomena are well documented for insect species in which one or several volatile organic molecules secreted from specialized exocrine glands elicit relatively stereotyped responses from conspecifics (2-5). It appears that similar chemical signaling occurs in

mammalian species (2-5), although the nature of the chemicals and the character of their communicatory function is poorly understood (2-5). Active chemicals have been denoted pheromones (6) and divided into subgroups of releaser and primer pheromones (7) but we, as others (8), question the use of this terminology, based primarily on insect studies, for mammalian communication because of chemical and behavioral complexity. For the rhesus monkey (Macaca mulatta), simple aliphatic acids ranging from acetic to isocaproic were identified from vaginal secretions and reported to elicit sexual responses from male conspecifics (9, 10). One component in a complex secretion from the tarsal scent gland of the black-tailed deer (Odocoileus hemoinus columbianus) has been characterized as cis-4-hydroxydodec-6-eneoic acid lactone and shown to play a role in sex, age, and individual recognition (11, 12). We report herein studies that establish a formidable complexity for urinary signals in guinea pigs.

BEHAVIORAL TESTING METHOD

In natural and seminatural circumstances, male guinea pigs respond differently to male and female conspecifics (13-15), and it is likely that these responses are mediated in part by chemosensory information. We found that adult sexually experienced male guinea pigs (Cavia porcellus) discriminate between male and female urine.

In a two-choice test, males were found to be more attracted by urine from intact non-receptive females, urine from adult males castrated at birth, and urine from ovariectomized females when each of these stimuli was compared with urine from intact males (16, 17). For the behavioral tests of urine fractions, two groups of sexually experienced adult males served as subjects. There were 12 animals in one group and 9 in the second. Each male subject was housed in a separate cage (55 x 50 x 35 cm high). Animals in both groups responded to fresh urine in a similar fashion, both before and after testing of the urine fractions; female urine was preferred to male

TABLE 1

ANALYSIS OF URINE AND URINE FRACTIONS

URINE SAMPLE TESTED			BEHAVIORAL RESPONSE		
			MEAN SECONDS		
METHOD OF SEPARATION			♀♀ fraction	♂♂ fraction	PROB.
None	Fresh urine before fractionation		139.0	33.3	<.01
Centrifugation	Supernatant		122.8	44.7	<.01
Dialysis against water	Non-dialyzable components		89.0	43.0	<.01
Chromatography					
	Urine filtrate	A	60.8	26.4	<.01
	Water wash	B	80.1	35.6	<.01
Amberlite XAD-2	Water wash	B-3	74.9	44.4	<.01
	Water wash	B-4	51.8	69.6	n.s.
	Ethanol eluate	C	73.1	13.6	<.02
Cation-exchange resin, fraction C	Filtrate	C-1	84.5	28.1	<.02
	Eluate with 1N NH_4OH in 65% ethanol	C-2	51.9	58.7	n.s.
Anion-exchange resin, fraction C-1	Filtrate	C-1a	96.0	37.9	<.02
	Eluate with 6N acetic acid in 65% ethanol	C-1b	72.9	33.3	<.05
Dialysis of fraction A against water	Non-dialyzable components		52.8	32.7	<.02
Separation by Volatility					
Sublimation of frozen urine	Volatiles		40.5	66.3	<.02
	Solid residue		81.2	26.9	<.05
Concentration of low-boiling components	Volatiles		32.7	58.3	<.01
	Liquid residue		62.1	11.1	<.05
None	Fresh urine after completion of the experiments		131.3	45.6	<.01

urine by all subjects (Table 1). Testing of the two groups alternated and no less than 48 hours separated connective tests of either group. No differences between groups in response to fresh urine or to urine extracts were evident. For each test, each animal was given a four-minute choice between a male urine fraction and a female urine

fraction. Extracts in organic solvents were vacuum evaporated to remove solvent. The residue was dissolved in ethanol and diluted with water. Maximal ethanol content was less than 1%. This concentration was shown to be without effect on test results with normal urine. For testing, all fractions were initially diluted to 50-100% of original urine volume with distilled water. Approximately 0.2 ml of each fraction was centered on each of two glass plates (7.5 x 15 cm, one plate containing a male urine fraction and the other containing a female urine fraction) and the two plates were placed in the animal's home cage with a 10 cm separation. The time spent by the male with his nose within approximately 1 cm of the urine or urine extract sample on each plate was recorded. All testing was conducted by a person unaware of the source of the samples. Clear-cut preferences for female urine compared to male urine were invariably accompanied by "head bobbing" and by physical contact of the nose with the urine. These acts might be involved in the mechanism by which large molecules are transported to chemoreceptors.

Urine samples were collected for 24 hours from 4 adult male and 4 adult non-receptive female (vaginal membranes intact) guinea pigs using metabolic cages. The urine was pooled every six hours and stored at 10°C. The animals were supplied with Rockland guinea pig diet and water ad lib.

RESULTS AND DISCUSSION

The different fractionation procedures for male or female urinary constituents and data from the behavioral tests are listed for each fraction in Table 1. Preliminary separation of highly polar, water soluble, and non-polar molecules was achieved by passing 200 ml of pooled, fresh urine (pre-centrifuged for 10 min. at 12,000 x g) through a 30 x 1.5 cm column of Amberlite XAD-2 (18, 19) which was pre-extracted with acetone for one day. The initial eluent (Fraction A) was active. The column was successively washed with four 50 ml portions of water giving fractions B-1 through

B-4. All four fractions combined (Fraction B) and Fraction B-3 alone were active, whereas the final wash (B-4) was inactive. Following this, elution of the column with 200 ml of ethanol gave a fraction (C) which was also active. This separation procedure clearly suggests that at least two components of the urine are involved in the discrimination.

Fraction C was further separated into acidic, basic, and neutral molecules by ion exchange chromatography. Fraction C was first concentrated by vacuum evaporation and then passed through a cation-exchange column (10 g, AG 50 W-X2, H^+ form). An ethanol wash (150 ml) gave Fraction C-1 which was active. Adsorbed, basic molecules were then removed from the column by elution with 1 N NH_4OH in 65% ethanol (40 ml). This eluent (Fraction C-2), which should contain phenolic amines (20), has shown no activity.

Fraction C-1, containing neutral and acidic molecules, was adjusted to pH 7.0 with 1 N NH_4OH and reduced in volume by vacuum evaporation. It was then passed through an anion-exchange column (10 g, AG 2-X8, acetate form) and washed through with 150 ml of 50% ethanol (Fraction C-1a). This fraction which should contain urinary conjugates and neutral molecules, including free steroids, was active. The column was then eluted with 6 N acetic acid in 65% ethanol to give fraction C-1b. Activity was also retained for this fraction containing the acidic urinary components.

After exhaustive dialysis of Fraction A (or of fresh male or female urine) against water in a cellulose tube at 0°C, the test animals showed a preference for the female sample. In a weighed amount of a freeze-dried sample of this fraction, protein was estimated by a microbiuret method (21) using bovine serum albumin as the standard. These values were close to the dry weight values, suggesting that this fraction consists essentially of proteins. This result strongly implicates a macromolecule or macromolecule-small molecule complex as being involved in chemosensory-based communication. Sex differences in urinary proteins are known in rats (22,

23) and it is possible that similar differences may occur in the guinea pig. These differences could account for the response differences in the behavioral test.

Fresh frozen male and female urine samples were sublimed, the volatiles collected, and the dry residues redissolved in water. The male guinea pigs still preferred the redissolved dry residue from the females compared with that from the males, while for the volatiles a preference for the male sample was observed. The same effect was noticed for the low boiling components of urine which were concentrated under vacuum (15 mm Hg) in a liquid nitrogen trap using a closed system. These results further illustrate the complexity of the chemical signals and associated behaviors.

The neutral, basic, and acidic fractions resulting from the ion-exchange chromatography were converted to their trimethylsilyl derivatives with Tri-Sil-"Z" after removal of the solvent. A comparative gas chromatographic (gc) analysis of these derivatives on a 3% OV 17 column (6' x 1/8", 100-270°C, 8°C/min., 30 ml He) was performed. Differences between male and female samples in gc-profiles were evident.

In a subsequent replication, active fractions from female urine (A, C, C-1, C-1a, and C-1b) were tested against water in the choice test. The female fractions were preferred in every case.

This study documents the complexity of urinary components that provide information on the sex of the animals. In contrast to the behavioral effect of a single compound or several closely related compounds as in many sex pheromones of insects (however, complexity of chemical communication for some insects is greater than was once thought [24]), the preference behavior of the male guinea pig for female urine appears to be mediated by a variety of chemically different components. The male-female urine discrimination is probably based on differences in the patterns of urinary components. These pattern differences most likely arise from sex differences in metabolism. For guinea pigs, and perhaps for many other species where urine plays a role in intraspecies communication, excreted or

secreted metabolic end products may have evolved a secondary, communicative role. The extreme specificity in the production and reception of chemical signals characteristic of many insect systems thus may be absent in urine-based mammalian chemosensory communication.

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