Role of the Vomeronasal Organ in Neonatal Offspring Recognition in Sheep

K.K. Booth and L.S. Katz

Department of Anatomy, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, Republic of South Africa

Department of Animal Sciences, Cook College, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08901-8525

ABSTRACT

Twenty-five pregnant Dorsett ewes were randomly divided into three groups to test if ewes use their vomeronasal organs for offspring recognition during nursing. One group of eight ewes (procaine) were made anosmic by irrigation of the nasal olfactory apparatus with a zinc sulphate procaine solution. The second group of nine ewes (cauterized) had their vomeronasal organs rendered nonfunctional by cautery of the nasoincissive duct. The third group of eight ewes were the controls. Parturition was synchronized in all ewes with betamethasone on Day 145 of gestation. Maternal responsiveness was tested two separate times with 1- to 2-day-old alien lambs. Each alien lamb trial was conducted 24 h apart. Cauterized ewes allowed alien lambs to suckle and they were unable to distinguish alien lambs from their own, whereas the ewes in both groups with functional vomeronasal organs (procaine and control) violently rejected any alien lamb’s attempt to suckle. Thus, female sheep use their vomeronasal organs for neonatal offspring recognition.

INTRODUCTION

Although visual [1-3] and auditory [4, 5] cues are used for offspring recognition at a distance, studies in a variety of mammals have demonstrated that olfaction is the primary cue used by female animals to recognize their offspring during nursing [6-22]. Anosmic goats were unable to distinguish their own kids from alien kids even though great variations in coat color were evident [23]. Blindfolded goats were able to distinguish their own offspring in a group of nonbleating kids [15]. Female goats required only 5 min of initial contact immediately after birth to discriminate their own kids from aliens after several hours of separation [15, 24]. Such quick recognition could not come from normal memorization of visual and/or auditory cues.

Offspring recognition via olfaction in most female animals is believed to be based upon the offspring’s individual olfactory signature [21, 25, 26], which is believed to emanate from its body coat [27, 28], but more specifically from the anal region [29]. Many of the odors from the anal region in animals have been associated with pheromones that are detected by the vomeronasal organ and that influence many types of animal activities from sexual maturation to maternal behavior [30-45].

The vomeronasal organ in sheep consists of two blind-ending, epithelial-lined tubes. The tubes are situated on either side of the base of the nasal septum and each is enclosed in a “C”-shaped cartilage [46]. The rostral portion of each tube opens into the nasoincissive duct, which communicates with both the nasal and oral cavities. The nasal cavity communication is on the floor of the nasal cavity, just caudal to the nasal vestibule. The oral cavity communication is on the incisive papillae, just caudal to the dental pad. One portion of the wall of the vomeronasal organ consists of sensory olfactory-like epithelium, while the remaining portion consists of typical respiratory-type epithelium. The sensory epithelium of the vomeronasal organ of sheep gives origin to nerves that pass through the cribiform plate of the ethmoid bone in close association with the nerves arising from the olfactory region of the nasal cavity [47]. The vomeronasal nerves bend around the dorsal margin of the olfactory bulb to reach the accessory olfactory bulb, which lies on the dorsal surface of the olfactory peduncle at the caudal margin of the olfactory bulb [47]. Nerve fibers of the accessory olfactory bulb project to the medial and cortical amygdaloid nuclei, from where fibers lead to the medial preoptic area and the medial hypothalamus. Thus, nerve fibers from the vomeronasal organ connect to areas of the brain identified with sexual behavior and the stimulation of gonadotropin production [48]. This pathway is different and separate from the nerves arising from the olfactory region of the nasal cavity [48]. Thus, volatile chemicals are detected by the olfactory epithelium in the caudodorsal region of the nasal cavity, while nonvolatile chemicals are detected by the olfactory-like epithelium in the vomeronasal organ.

A method by which nonvolatile chemicals are delivered to the vomeronasal organ is demonstrated during the well-known lip curl or flehmen behavior used by ruminants during urination analysis. In the procedure, a sample of urine is delivered via the tongue to the incisive papillae. Then the fluid is sucked through the incisive duct to the vomeronasal organ as the curled lips block the external nasal openings [48]. In addition to the investigation of urine, nonvolatile dyes have also been shown to enter the vomeronasal organ during such activities as self grooming, social grooming, and consummatory behavior [49]. Because grooming by licking is one of the first contacts a mother animal has with her newborn, it is quite possible that nonvolatile chemicals are delivered to the vomeronasal organ via the incisive papillae from either the skin or the amniotic fluid, which covers the offspring at birth. Removal of the amniotic fluid from lambs before ewes had the chance to clean their offspring disrupted the development of the initial mother-offspring bond [50]. A neonatal pheromone could explain the individual olfactory signature theory [21, 25, 26], as well as the very short contact period necessary for female goats to discriminate their own from alien kids [15, 24]. Thus,
the purpose of this investigation was to determine if the vomeronasal organ is used as a determinant for neonatal offspring recognition in sheep.

MATERIALS AND METHODS

Animals

All research with animals was approved by the Rutgers University Animal Care and Facilities Committee. Estrus was synchronized in 30 Dorsett ewes with two i.m. injections of lutalyse (10 mg: Pharmacia & Upjohn Co., Kalama-zoo, MI) administered 10 days apart. The ewes were mated 1 day after the second injection and then randomly divided into three groups.

Ten ewes assigned to the procaine group were rendered anosmic by two irrigations of the nasal olfactory mucosa with a solution of zinc sulphate (1.5%) (Sigma, St. Louis, MO) and procaine (3%) (Sigma) as described by Poindron [19, 51] and Chemineau et al. [52]. The first irrigation occurred at 4 wk and the second irrigation occurred at 3 wk before parturition. Anesthesia was produced in each ewe with halothane delivered through an inhalation cone placed over the nose and mouth. The animal's nasal region was elevated to a 75° angle to the horizontal. The zinc sulphate-procaine solution was delivered by a 30-cm-long atomizing catheter, which was connected to a 5-ml syringe. The solution-filled catheter was passed carefully through the external nasal opening, through the dorsal nasal meatus, and to the caudal limit of the olfactory epithelium, which is situated in the caudal-dorsal region in both the right and left sides of the nasal cavity. After delivery of 1 ml of solution at this point, the catheter was withdrawn 5 cm and another 1 ml of solution was administered to ensure coverage of the entire olfactory epithelium. A few seconds after each delivery of the zinc-sulphate-procaine solution, each ewe would demonstrate a swallowing reflex, indicating ingestion of excess solution. There were no indications of any side effects from this small amount of ingested solution in any ewe. No ewe showed any signs of solution aspiration into the lungs. After irrigation of the olfactory region in each side of the nasal cavity was completed, the nasal region of each animal was maintained at the 75° angle for 15 min to ensure that any excess zinc sulphate-procaine solution drained caudally toward the pharynx. Thus, the solution did not come into contact with the olfactory epithelium of the vomeronasal organ through the organ’s nasal opening, which is situated at the rostral end of the floor of the nasal cavity. The efficacy of this procedure was tested by breakage of an ammonia ampule in front of the conscious animal’s nostrils. Although the test of avoidance of food soiled with dog feces or urine may have been a better test of anosmia, the absence of any reaction to the broken ammonia ampule in the treated animals versus the immediate avoidance of the ampule in nontreated animals was considered an adequate test of the anosmic condition in the procaine-treated group. This procedure produced a group of ewes that were unable to detect volatile chemical odors; however, the nonvolatile chemical detecting epithelium within their vomeronasal organs remained functional. At the time of lambing, one ewe was not pregnant and one other ewe delivered a stillborn lamb. Thus, a total of eight ewes were tested in this group.

Ten control ewes were anesthetized as in the previous two groups; however, their nasal cavities were irrigated only with 2 ml of normal saline solution. Thus, both the normal odor detection system as well as the vomeronasal system remained functional in this group of ewes. At the time of lambing, two ewes delivered stillborns. Thus, only eight ewes were tested in this group.

Initially, the ewes in each group were housed together and given free access to food and water. One month before scheduled lambing, each group of ewes was housed in separate facilities. Seven days prior to their scheduled parturition, each ewe was placed in a separate birthing pen (2 m width, 3 m length) within their grouped housing. Parturition was induced by i.m. injection of 20 mg betamethasone (Sigma) on the evening of gestation, Day 144, followed by 10 mg betamethasone on the morning of gestation, Day 145 (if necessary). All lambs were marked immediately after birth with a colored grease stick. A numerical marking was placed over their backs and corresponded to the number on the back of the ewe. The lambs remained with their dams at least until the end of the first suckling episode and then the lambs were isolated from their dams for a minimum of 4 h to stimulate hunger and teat-seeking activities.

Behavioral Testing

Each ewe received a 1- to 2-day-old alien lamb equal in size to her own offspring. The alien lambs were taken from different group housing facilities to ensure that ewes and lambs were out of hearing range of each other. The following behavioral recordings were collected: number of low-pitched, closed-mouth (maternal interest) bleats; number of high-pitched, opened-mouth (maternal distress) bleats; number of smelling episodes (consisted of ewe sniffing and making muzzle contact with lamb’s body); number of tongue manipulations of the palate (ewe licks her own muzzle after making contact, usually with the lamb’s anal region, and running her tongue over the incisive papillae); number of udder acceptances (lamb’s head engaged under ewe’s inguinal region and ewe did not move for 10 sec); number of udder rejections (ewe moved away from lamb within 10 sec after lamb was engaged under her inguinal region); number of suckling episodes of 5-sec duration; number of aggressive behaviors (threats, butts, bites, or kicks); number of changes of position (ewe either avoided
lamb’s approach to the udder or ewe circled around lamb as it tried to reach the inguinal region).

To prevent injury to the lamb, rejection was considered positive if a minimum of two forceful butts (aggressive behavior) were delivered. The lamb was then immediately removed. The duration of the behavior test was at least 10 min unless the ewe demonstrated aggressive behavior toward the lamb. This test was repeated 24 h later using the same lamb or lambs (in the case of twins) as in the first test. Thus, behavioral observations were collected for two trials on each ewe. Assistants who were familiar with acceptance/rejection behavior, but not familiar with the assignment of treatments, recorded the data.

**Statistical Analysis**

The data were analyzed using the GLM procedure of the SAS system (Version 6.11, 1989–1996) with treatment and trial as the independent variables. Probabilities less than 0.05 were considered significant. Mean comparisons were evaluated by the Bonferroni test. Within a treatment group, data for the two behavior trials did not differ. Thus, a single mean value for each behavior frequency was calculated for each ewe. Results are presented as mean ± SEM.

**RESULTS**

Ewes in the cauterized group emitted more low-pitched bleats than either control or anosmic ewes ($P < 0.05$; Fig. 1). High-pitched bleat frequency did not differ from the controls in either the procaine or cauterized groups. Ewes with cauterized nasoincisive ducts spent more time sniffing the lambs and also manipulated their palates more frequently than the ewes in the other groups ($P < 0.05$; Fig. 2). The total sucking time was greater for ewes with cauterized nasoincisive ducts (63.8 ± 5.8) than for ewes with procaine-treated olfactory epithelium (0.6 ± 0.6) or controls (0.9 ± 0.9; $P < 0.05$; Fig. 3). Frequency of aggressive behaviors directed at the alien lambs differed among the three groups with procaine the highest and cauterized the lowest ($P < 0.05$; Fig. 3). Changes of position of the ewe, indicative of the ewe avoiding the lamb’s approaches, were lower in the cauterized group than in controls ($P < 0.05$; Fig. 3). Control ewes and procaine-treated ewes never accepted the lambs at the udder, whereas cauterized ewes allowed the alien lambs to suckle for long periods of time ($P < 0.05$; Fig. 4). Rejection of lambs at the udder was lower for the cauterized ewes than for the other two groups ($P < 0.05$; Fig. 4). All ewes in each group accepted their own lambs at the udder when the lambs were returned to their mothers after each trial.

**DISCUSSION**

The control ewes, in which both the nasal olfactory apparatus and the vomeronasal organ were functional, demonstrated typical alien lamb rejection behavior. As soon as the lambs tried to engage in sucking at the udder, the control ewes would sniff the lambs’ anal regions, lick their muzzles, make tongue manipulations along the palate, and immediately move away from the lambs. If the alien lambs...
persisted in their attempts at the udder, the ewes would become aggressive and butt the lambs. The control ewes changed positions in an attempt to get away from the alien lambs and rejected the alien lambs at the udder more often than the ewes in the cauterized group.

Similar behavioral patterns were observed in the procaine group of ewes, in which the nasal olfactory apparatus was rendered nonfunctional, but the vomeronasal organs remained functional. This group of ewes rejected alien lambs equally as the ewes in the control group, even though the normal sense of smell through their nasal olfactory apparatus was blocked. This behavior pattern does appear to be in contrast to those studies in which a functional nasal olfactory apparatus was determined to be necessary for offspring recognition [6–8, 16, 18, 19, 21]; however, it is very possible that the procedures employed in these previous studies to eliminate the function of the nasal olfactory apparatus also eliminated the function of the vomeronal organ. For example, because of the intimate relationship of the vomeronal nerves to the olfactory bulb and the olfactory peduncle, ablation of the olfactory bulb by either chemical or physical means could interrupt the function of the vomeronasal organ. If care is not exercised to prevent excess zinc sulphate from draining out of the nasal cavity through the nostrils, the chemical agent could easily gain access to the vomeronal organ through the nasal cavity of the nostril. The chemical agent could easily gain access to the vomeronal organ through the nasal cavity of the nostril. The chemical agent could easily gain access to the vomeronal organ through the nasal cavity of the nostril. The chemical agent could easily gain access to the vomeronal organ through the nasal cavity of the nostril.

The only measured behavioral expression that was significantly different between the procaine-treated and control ewes was aggression. The increased aggression in the procaine group may be a result of heightening vomeronal organ discriminatory function following the loss of normal nasal olfaction.

Even though cauterized ewes had full use of all other senses that have been reported as important for offspring recognition [2–7, 16, 17, 19, 23, 27], they acted differently toward alien lambs than the ewes with functional vomeronal organs. The cauterized ewes demonstrated more interest in the alien lambs, as was indicated by the higher number of low-pitched bleats in this group when compared with the other two groups. As the alien lambs tried to engage in suckling at the udder, these ewes would also sniff the lambs’ anal regions and also manipulated their palates with their tongues as in the other groups. However, the cauterized ewes behaved as though they were confused and could not detect any recognition signal. This was evident by their continued sniffing of the lambs’ anal region and repeated tongue manipulations during the entire trial period. These behavior patterns were significantly higher in this group of cauterized ewes. As a result of their inability to
detect a recognition signal through their vomeronasal organs, the cauterized ewes displayed significantly less aggression, less rejections, more acceptances to the udder, and allowed alien lambs to suckle longer than the ewes in the other two groups with functional vomeronasal organs. Also, when lambs were returned to their mothers after each trial, the ewes in every group accepted their own lambs at the udder. This was especially evident in the normal ewes and the procaine-treated ewes. The cauterized ewes also accepted their own lambs; however, the initial behavior of these ewes toward their own lambs was identical to that demonstrated toward alien lambs. Repeated sniffing of their own lamb’s anal region, repeated licking of their muzzle, and repeated tongue manipulations of the palate further demonstrated their inability to detect a recognition signal. Thus, it is concluded that the vomeronasal organ is used as the confirming determinant for neonatal offspring recognition during nursing in sheep.

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