Control of Secretion From the Avian Salt Gland

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ABSTRACT

FÄNGE, RAGNAR, KNUT SCHMIDT-NIELSEN AND MARYANNE ROBINSON, Control of secretion from the avian salt gland. Am. J. Physiol. 195(2): 321-326. 1958.—In birds with a marine habitat the nasal glands are modified into salt glands, able to excrete excess sodium chloride. The nervous control of the salt glands was investigated in the herring gull, Larus argentatus. Normal secretion of the salt gland seems to be released by a nerve reflex involving higher nerve centers and central osmoreceptors. The reflex can be evoked experimentally by intravenous injection of a hypertonic NaCl solution or by increasing the osmotic pressure of the blood in other ways. The gland is innervated from a nerve plexus in the anterior part of the orbit of the eye. Secretion is produced by stimulation of a nerve, probably a branch of the VII cranial nerve, which connects with the plexus. The plexus also receives sympathetic fibers, but no secretion was observed after stimulation of the cervical sympathetic. The gland is stimulated to secrete its osmotically highly concentrated fluid (700-800 mM Na) by Mecholyl and acetylcholine, indicating a parasympathetic innervation as the normal excitatory pathway. The secretion that normally occurs in response to a salt load is blocked by anesthesia. It is also inhibited by atropine, adrenaline or acetazolamide (Diamox).

Although the nasal gland (glandula lateralis nasi) was described as early as in 1664 by Nicolaus Steno (1), it has been neglected by physiologists, probably because in man it exists only in the embryological stage (2). Nasal glands are found in birds, reptiles and most mammals. They usually are tubulous and serous, and their function is supposed to be that of moistening and protecting the nasal membranes. However, in birds with a marine habitat (gull, albatross, pelican, penguin, etc.), the nasal gland is modified into a remarkably efficient organ for excretion of excess sodium chloride from the body. This salt gland has an interesting microscopic structure somewhat reminiscent of the medulla of the mammalian kidney, consisting of closely packed parallel secretory tubules surrounded by blood capillaries. A description of the anatomy and microscopic structure of the salt gland of the herring gull is given by Fänge, Schmidt-Nielsen and Osaki (2b). The secretory fluid from the salt gland of marine birds and reptiles has a salt concentration many times as high as that of the blood, and often nearly twice as high as that of sea water (3-5). Since the avian and reptilian kidneys cannot produce a very concentrated urine, the salt gland is essential in the excretion of salts from sea water ingested with the food or for drinking (6).

Normally the salt gland secretes only when there is an osmotic stimulus, i.e. after absorption of sodium chloride from food or water with a high salt content. The secretory response is probably brought about by a nerve reflex, supposedly involving osmoreceptors situated in the brain or in the wall of certain blood vessels. It has been the purpose of the present investigation to study if, and to what extent, the salt gland is controlled by nerve action. A secretory nerve is described, and the effects on the gland of nerve stimulation and of different drugs are reported (see also 7).

MATERIAL AND METHODS

Material. Young specimens of herring gull (Larus argentatus) were trapped and kept in
Approach for cannulation of the salt gland duct, showing the palate from below. The left half of the drawing shows the location of the incision. In the right half of the drawing a part of the roof of the mouth is removed, exposing the region of the vestibular concha with the two openings of the ducts from the salt gland. S.G.: openings from the supramaxillary glands.

Operation Technique. In most cases chloral hydrate was used for anesthesia. It was given intraperitoneally in a solution obtained by dissolving 1 gm chloral hydrate in 1 ml water. After an initial dose of 0.3 ml, one or two further doses of 0.1 ml were added, if necessary, to obtain the desired degree of anesthesia. Intravenous injection of Nembutal was used in some cases. During the operation the animals were loosely wrapped in cheese cloth and fixed to an operating table. Exposure of the secretory nerve was made by removing the eyelids and pulling the eye aside or entirely removing it. The major nerves of the orbit could then be found without difficulty (see description later in the text). The cervical sympathetic, which in birds runs in the foramen transversum of the cervical vertebrae, is difficult to reach. The operation to expose it was made according to Langley (8). Cannulation of the gland ducts was made through incisions made in the palate (fig. 1), by means of polyethylene tubings, 0.8 mm o.d., which were introduced into the lateral duct openings. Attempts to cannulate the median duct openings in a similar way were unsuccessful.

Nerve Stimulation. Before stimulating the secretory nerve it was cut as far centrally as possible. Stimulation was given by silver electrodes from a thyatron stimulator. In most experiments the frequency was 20/sec. at 10 volts intensity.

Osmotic Load. About 10 ml/kg body weight of 10% NaCl solution were injected into a vein on the median side of the foot.

Drugs Used. The drugs used were: acetyl choline chloride, methacholine chloride (Mecholly), atropine sulfate, l-adrenaline chloride, histamine phosphate, pilocarpine hydrochloride and acetazolamide (Diamox). The doses given in the text refer to the amount of the salts injected per kilogram bird.

Collection of Secreted Fluid. Samples were either collected from the cannulated lateral duct or as drops from the tip of the beak. In the former case the sample came from about one-fourth of the gland, while in the latter case the total secretion from the salt gland was collected. Usually samples were collected in five minute periods. Analyses of sodium, potassium and chloride were performed as in previous work (3).

RESULTS

Osmotic Stimulation. The normal stimulus for secretion is an increase in plasma salt concentration. In nonanesthetized birds such stimulus can be given by intravenous injection of for example 10 ml 10% NaCl solution/kg body weight. This caused secretion of large amounts of fluid from the salt gland. The first secretion appeared 1–5 minutes after the injection. High secretory rates could persist for several hours after a heavy salt load. During this time the gland secreted at a rate of 0.3–0.4 ml/min/gm gland tissue. The osmotic concentration of the secretion was always very high (700–800 mM Na).

In anesthetized animals the secretion after a similar salt load was delayed 1 or 2 hours and
SECRETION FROM THE AVIAN SALT GLAND

did not start until the narcosis began to wear off. Then a secretion of the same kind as observed in nonanesthetized birds began. It is well known that different narcotics have a blocking effect on salivary secretion in mammals (9). In work with the submaxillary gland of cat, chloralose is often used as an anesthetic because it affects the secretion less than other substances. In a few cases in which we tried chloralose on gulls it gave an undesirable condition of epileptiform cramps.

Innervation. According to Gaupp (10) and Marples (11) the avian nasal gland is reached by branches from the ramus ophthalmicus of the nervus trigeminus. Also fibers from the ramus maxillaris of the nervus trigeminus passing along the posterior orbital wall are reported to go to the nasal gland (10–12). Other authors have found that the gland is innervated from a ganglion in the anterior orbital region, the ganglion being called ganglion ethmoidale (12, 13) or ganglion sphenopalatinum (14). The ganglion, which also innervates the Harderian gland, is described to have connections with the nervus trigeminus, the nervus facialis and the sympathetic system. Cords (12) also mentions a connection with the nervus glossopharyngeus. The study of the anatomical literature gives the impression that the parasympathetic nerves and ganglia in the head of birds still are imperfectly known anatomically and that there is considerable uncertainty and confusion concerning the terminology.

In the gull the salt gland is situated on the top of the skull and forms a crescent-shaped mass above each eye. Nerves reach the gland from the orbit. In order to find the secretory nerve to the salt gland, we made dissections and stimulated electrically different nerves, which were prepared free, in narcotized birds. It was found that a thin nerve in the anterior, median part of the orbit is the secretory nerve of the salt gland. A suitable method for finding the nerve is to pull the eye backward and downward after sectioning of the upper eye muscles. This exposes the large Harderian gland between the eye bulb and the anterior edge of the orbit (fig. 2). When the margin of the gland is followed into the orbit, a large nerve (ramus ophthalmicus) is seen, which stretches in a horizontal direction. Finally joining the large nerve there is a much finer nerve which closely adheres to the upper margin of the Harderian gland. This is the secretory nerve. At the place where the two nerves meet, a plexus containing numerous ganglion cells is found. From this plexus or ganglion fine, apparently nonmyelinated fibers pass into the anterior part of the gland.

By dissection, the secretory nerve could be followed backwards and downwards to the tympanic region, where it seemed to form a connection with nervus facialis. Probably the secretory nerve and the ganglionic plexus correspond to the ramus palatinus of nervus facialis and ganglion ethmoidale (12).

No secretion was observed after stimulation of ramus ophthalmicus or after stimulation of a thin nerve which follows the posterior wall of the orbit, nor was any secretion observed when the cervical sympathetic chain was stimulated. In the latter case a movement of the feather muscles on the top of the head was observed in accordance with Langley (8), indicating a successful stimulation of the cervical chain. The same sympathetic reaction was also occasionally observed when the secretory nerve was stimulated, indicating that this contains some sympathetic fibres as well.

Nerve Stimulation. When the secretory nerve was stimulated secreted fluid appeared in the cannulated lateral duct of the same side of the head within 1/2–1 minute. In the beginning the secretion was slow, but after continuous stimulation for 5–10 minutes, fluid dropped
from the cannula at a rate of 0.05 ml/min. which corresponds to 0.3 ml/min/gm gland tissue. Thus, during nerve stimulation the gland was secreting at about the same rate as after a salt load (0.3-0.4 ml/min/gm gland tissue).

When the frequency of stimulation was lowered from 20/sec. to 2/sec. the secretion almost stopped (fig. 3), but increased again with increase of frequency. When stimulation was interrupted, the gland continued to produce secretion for several minutes (fig. 3).

The electrolyte concentration of the secreted fluid was of the same magnitude as that obtained after a salt load (ab. 700-800 mEq/l. Na). In a few cases the surface of the gland was observed by means of low power binocular microscope during nerve stimulation. There occurred a vasodilatation during stimulation, but it was not possible to observe which vessels reacted.

Mecholyl. Mecholyl (0.1-0.5 mg) injected intravenously produced a secretion that lasted for several minutes in both nonanesthetized and anesthetized birds. Since Mecholyl stimulates several glands, the secretion collected by a glass tube held below the beak of the birds is large in volume and more dilute (200-500 mEq/l. Na) than the pure secretion from the salt gland. Much more concentrated secretion (500 800 mEq/l.) was obtained from cannulas in the lateral duct.

Acetylcholine. Intravenous injection of 0.1 mg acetylcholine had no effect on the secretion while 0.5 mg produced one drop of secretion. This latter dose had strong general effects on respiration etc. Much smaller doses of acetylcholine injected into the carotid artery (Nembutal-anesthetized bird) produced secretion from the salt gland. While intra-arterial injection of 0.001 mg acetylcholine produced secretion only from the gland of the same side of the head as the injected artery, a dose of 0.01 mg acetylcholine produced secretion from the salt gland on both sides of the head.

Pilocarpine and Histamine. In nonanesthetized birds 3 mg pilocarpine intravenously had no observable effect on the salt gland, but
other glands (probably the supramaxillary glands and small mucous glands) produced liberal amounts of a mucous secretion. Small doses of pilocarpine (0.05 mg and 0.3 mg) or histamine (0.05 mg and 0.2 mg) had no secretory effects.

**Atropine.** A dose of 0.01 mg of atropine injected intravenously reduced the secretion produced by a salt load, and 0.1 mg practically abolished the secretion (fig. 3). Secretion produced by nerve stimulation was also blocked by atropine. The slight secretion still going on in spite of atropine had a somewhat lower concentration of sodium than the normal secretion after a salt load.

**Adrenaline.** When given in a slow intravenous injection epinephrine (0.2 mg) caused a transient block of secretion (fig. 3), perhaps due to its vasomotor effect. The osmotic concentration of the fluid secreted in one case increased a little after adrenaline, but in another animal a slight decrease in Na concentration was observed.

**Diamox.** Intravenous injection of this carbonic anhydrase inhibitor had a distinct inhibitory effect (fig. 3), but a subsequent dose of Mecholyl started the secretion again in spite of the presence of Diamox. The experiment was repeated twice with the same result. An analyzed sample showed a sodium concentration of about 150 mEq (noncannulated glands), which shows that the secretion was produced by the salt glands. Since the gland is able to produce the usual high ionic concentration in the presence of Diamox, one can assume that the blocking effect does not consist in interference with an ionic transport mechanism in the gland.

**Secretion From Other Glands.** Under certain stimuli activity was noticed in other head glands which could influence the volume and composition of collections from the beak. Therefore, the secretion from two of these other glands was briefly studied. The Harderian glands, with a total weight of more than 1 gm, are larger than the salt glands. They seem to produce tear fluid (5). A copious secretion from the Harderian glands was obtained after Mecholyl (0.1–0.5 mg) intravenously. The supramaxillary gland (15) is a pair of tubiform glands near the tip of the beak. They were found to secrete after pilocarpine (3 mg) or Mecholyl (0.1–0.5 mg) intravenously, but could also be stimulated reflexively by slightly scratching the palate around the openings. Samples of secretion collected by polyethylene catheters from the Harderian and the supramaxillary glands after Mecholyl were analyzed for sodium and potassium.

The concentrations of sodium were found to be: salt gland: 520–846, Harderian gland: 154–169, and supramaxillary gland: 176–196 mEq Na/l.

The potassium concentrations for the same three glands were: salt gland: 10–40, Harderian gland: 10, supramaxillary gland 1 mEq K/l.

The approximate plasma concentrations of sodium and potassium in the herring gull are 160 mEq and 5 mm, respectively. Thus the secretion from the Harderian gland is about isotonic, and that from the supramaxillary glands slightly hypertonic in respect to sodium concentration in the blood, while that from the salt gland is highly hypertonic. It is obvious that the relatively low osmotic concentration of the secretion collected from the beak after Mecholyl injection can be explained as a dilution with the secretions from the Harderian and supramaxillary glands.

**DISCUSSION**

If the osmotic concentration of the blood is increased by uptake of either sodium chloride from the intestine, or by intravenous infusion of hypertonic solutions of sodium chloride or sucrose, the salt gland, apparently due to a nerve reflex, begins to secrete. The blocking of the secretory reflex by narcosis may indicate

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**Table 1. Physiological responses of the salt gland**

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<thead>
<tr>
<th>Normal stimulus</th>
<th>Salt load</th>
<th>Secretion</th>
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<tr>
<td>Other osmotic stimulus</td>
<td>Sucrose</td>
<td>Secretion</td>
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<tr>
<td>Nerve stimulation</td>
<td>Secretory nerve</td>
<td>Secretion</td>
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<td></td>
<td>R. ophthalmicus</td>
<td>No secretion</td>
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<td></td>
<td>Cervical sympathetic</td>
<td>No secretion</td>
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<td>Parasympathomimetic drugs</td>
<td>Acetylcholine</td>
<td>Secretion</td>
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<td></td>
<td>Mecholyl</td>
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<td></td>
<td>Pilocarpine</td>
<td>No secretion</td>
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<td>Other substances</td>
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<td></td>
<td>Nembutal</td>
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the involvement of higher nerve centers in the reflex. Nerve stimulation as well as the effects of various drugs, as summarized in table 1, indicate a parasympathetic nature of the innervation.

The secretory nerve to the salt gland is probably a branch of nervus facialis, but due to the complexity of ganglia and nerve anastomoses in the head, this is difficult to determine with certainty. Postganglionic, probably cholinergic, fibers pass to the gland from a nerve plexus (ganglion ethmoidale) in the anterior part of the orbit. Undoubtedly the secretory nerve, besides parasympathetic fibers, also contains sympathetic fibers passing to the skin of the head. It is not clear whether there is any sympathetic innervation of the salt gland. Stimulation of the cervical sympathetic did not cause any secretion from the gland, and adrenaline injection had a blocking effect. The blocking effect from adrenaline can be due to a temporary vasoconstriction in the gland, while the blocking by carbonic anhydrase inhibitor (Diamox) is more difficult to explain. It does not seem to be due to an inhibition of carbonic anhydrase in the gland cells, because these can still work if stimulated by Mecholyl. Therefore, it is probable that Diamox exerts its blocking effect on some part of the reflex chain that normally conveys the stimulus from the hypothetical central osmoreceptor system to the gland.

In experiments with the salt gland in the gull it is necessary to take into consideration the possibility that the secretory samples are contaminated by the secretion from the Har-derian gland or the supramaxillary glands. Some other smaller glands might also interfere. However, among all the glands in the head of the gull the salt gland seems to be the only one which is stimulated to secrete by an osmotic load.

REFERENCES

1. STENO (STENSEN), N. De musculi et glandulis. Amstelodami 1664 (quoted from: BROMAN 1921).
8. LANGLEY, J. N. J. Physiol. 30: 221, 1903.