Comparison of the Effects of Parasympathetic and Sympathetic Nervous Stimulation on Cat Submaxillary Gland Saliva* (33545)

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(Introduced by S. C. Wang)

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It is generally agreed that all major mammalian salivary glands receive parasympathetic secretory innervation. The secretory role of the sympathetic system has been more controversial. Stimulation of the sympathetic nerves to some glands, such as the cat parotid, yields little or no salivary flow whereas this procedure elicits a relatively copious flow from other glands, such as the cat submaxillary. However, even in this well studied gland, the contributions of each autonomic system to the total secretion are still not well understood. Most previous studies have focused primarily on differences in flow rate characteristics and, to a much more limited extent, on relative differences in concentration of sodium, potassium, and total protein [(1), for review]. In addition, in many studies, drugs were used in an attempt to mimic nervous stimulation of salivary flow. More specific secretory roles for each system might be established if qualitative, as well as marked quantitative, differences were demonstrable in salivary content when each nerve was stimulated directly. Only Komarov and Stavraky (2) have indicated that protein secreted in each type of saliva from the cat submaxillary gland appeared to have different chemical and physical properties. In the present study we have attempted to determine if there were identifiable protein fractions unique to one type of saliva as well as to study differences in the ionic and nonionic constituents.

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Methods. Thirteen cats, of both sexes, weighing 2.7–3.2 kg, were anesthetized with a mixture of chloralose, 30–40 mg/kg, and urethane, 400 mg/kg, administered intraperitoneally. The animals' rectal temperature was maintained between 37 and 38.5° with heating pads. Both submaxillary ducts were cannulated with lengths of polyethylene tubing (PE 10) which were tied in place.

The branch of the chorda-lingual nerve to the submaxillary gland and the cervical sympathetic trunk on the same side were dissected free and cut centrally. The nerves were stimulated at rates of 2–20 Hz with square pulses having a 1.0-msec duration, and a 1.0 mA current intensity. In 3 experiments the nerves were stimulated continuously and in the other 10 there were alternating 30-sec periods of stimulation and rest until a 1.5-ml sample was collected. Samples were collected during stimulation of (i) sympathetic nerve, (ii) parasympathetic nerve, and (iii) both nerves at the same time. Approximate rates of flow were calculated by dividing the volume of the sample collected by the total collection time.

Each sample was examined by two electrophoretic methods. One was vertical disc electrophoresis employing the method of Ornstein and Davis (3). Samples were run in 7.5% polyacrylamide gel and Tris–glycine buffer, 0.1 *M*, pH 8.3, and separation was obtained by applying a current of 5 mA per tube for about 1 hr. Samples were stained with Coomassie brilliant blue. Gels prepared from samples obtained from the same animal had equivalent amounts, 30 or 50 μg, of total protein. A second method used was flat-bed electrophoresis. This technique, a microadaptation (Ellison, unpublished) of the procedure described by Meyer and Lamberts (4), utilized

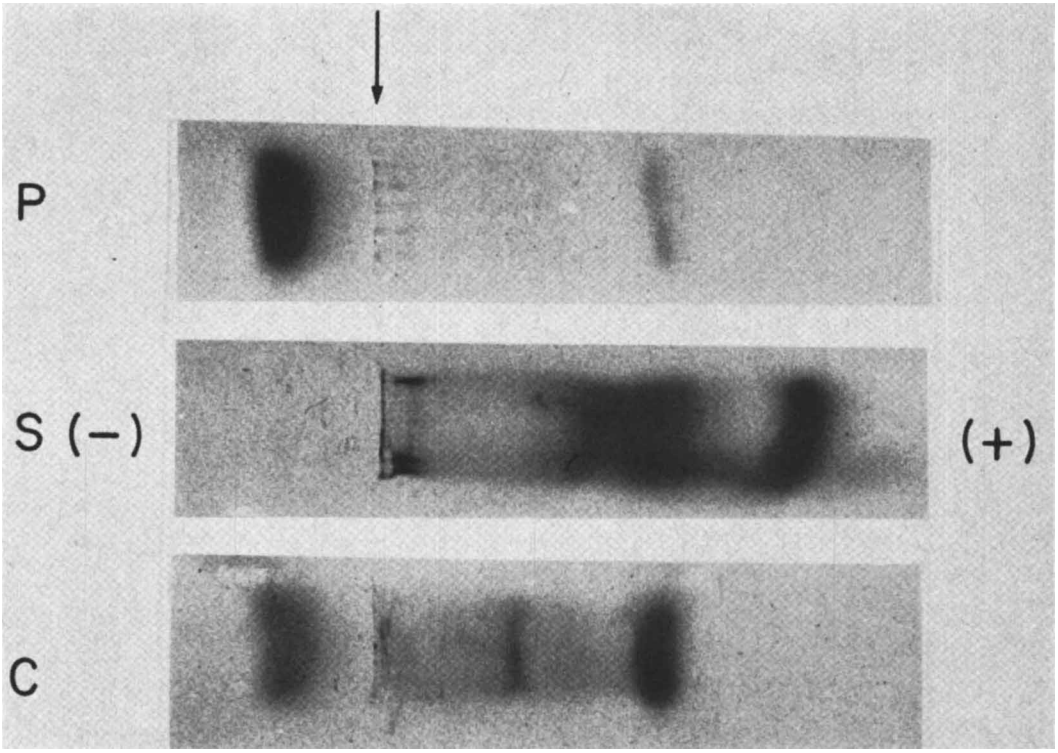

FIG. 1. Flat-bed electrophoresis: (top to bottom) parasympathetic (P); sympathetic (S); and combined (C); arrow marks point of application. Note the absence of any stainable cationic substance in the sympathetic sample.

5% acrylamide gel, 0.05 *M* barbital buffer at pH 8.6 and Coomassie brilliant blue stain. Glycoproteins were stained with the periodic acid–Schiff technique of Keyser (5).

Sodium and potassium were measured by atomic absorption spectrophotometry. Calcium was analyzed by this method, using lanthanum chloride as a diluent. Urea nitrogen was determined by the indophenol method after hydrolysis with urease (6). Total protein was estimated by the Folin–Ciocalteu method (7). Fucose was determined by the primary cysteine reaction of Dische (8).

As there was wide variability in the concentrations of salivary contents between individuals, we used each experiment as its own control in the statistical evaluation of the data. We calculated differences between samples collected from the same animal during the three conditions of stimulation and determined the level of significance of the differences between the means of each group (9).

Results. Flat-bed electrophoresis. The cationic components of the three different types of samples were studied with flat-bed electrophoresis. This yielded a very straightforward and dramatic result. All the parasympathetic samples exhibited a heavily staining component which migrated toward the cathode. This cationic substance was present to a lesser extent in the combined and was absent in the sympathetic samples[2] (Fig. 1). This component did not stain positively with the PAS technique.

Vertical disc electrophoresis. This technique revealed quantitative and, possibly, qualitative differences between the anionic components of the sympathetic and parasympathetic samples (Fig. 2). In all of the sympathetic samples there were one to three rapidly

[2] T. S. Meyer, Naval Dental Research Institute, Great Lakes, Illinois, using a different electrophoresis technique, obtained similar results with samples provided by the authors.

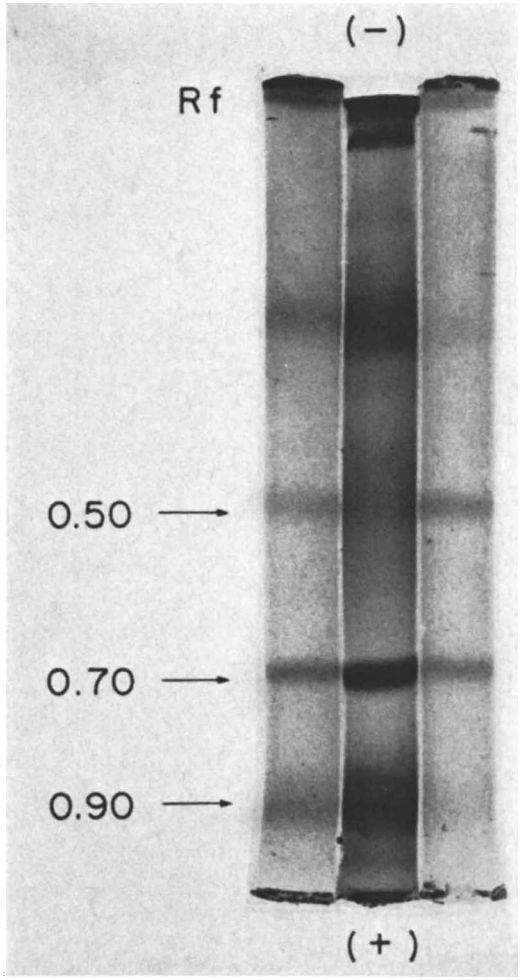

FIG. 2. Vertical disc electrophoresis: Left to right: Combined; Sympathetic; and Parasympathetic. R_f Values are on the left.

migrating components having R_f values from 0.86 to 0.93. Banding in this region occurred in only 4 of 13 parasympathetic samples and the staining was much less intense than in sympathetic samples from the same animals. These rapidly migrating components yielded a positive PAS reaction, indicating their glycoprotein nature.

A diffusely staining area, R_f 0.40–0.60, was found in many of the sympathetic and none of the parasympathetic samples. Further study of this stainable material is now underway.

One band appeared consistently in every sample of each type (R_f = 0.70–0.72). It, too, was PAS positive.

Flow rates and chemical analyses. The flow rate during sympathetic stimulation was 0.10 ± 0.06 ml/min (mean ± SD). The rates during parasympathetic and combined stimulation were 0.18 ± 0.13 and 0.20 ± 0.14 ml/min, respectively. A summary of the chemical analyses is presented in Table I.

Potassium concentration was significantly higher in the sympathetic than in the parasympathetic and combined samples ($p<.001$ for both). Sodium concentration was higher in the sympathetic and combined than in the parasympathetic samples ($p<.001$ and $<.005$). The average parasympathetic flow rate was 1.8 times that of the sympathetic and the average combined flow rate was 1.1 times that of the parasympathetic. However, even in four cases where the parasympathetic flow rate exceeded that of the combined, the combined [Na] exceeded that of the parasympathetic.

Sympathetic [Ca] was higher than the parasympathetic and combined calcium levels ($p<.05$ for both). Urea nitrogen was higher ($p<.01$) in the sympathetic than in the parasympathetic collection, but there was no difference between these and the combined samples. There were no significant differences found between the levels of the total protein and fucose in the three different types of samples.

Discussion. The results of the present study indicate that there are demonstrable qualitative differences between the protein constituents of the parasympathetic and sympathetic saliva secreted by the cat submaxillary gland. In the parasympathetic saliva there are cationic proteins, not found in the sympathetic secretion, which are probably not glycoprotein in nature. These constitute a large fraction of the total protein secreted in parasympathetic saliva. The presence of these proteins in one type of secretion and not the other indicates that different secretory processes are involved in the formation of each secretion.

On the basis of these experiments it is not clear whether these proteins are excreted

TABLE I. Salivary Contents (mg/100 ml; mean ± SD).

Stimulation	K	Na	Ca	Urea N	Fucose	Folin
Sympathetic ($N = 13$)	65.4 ± 16.4	109.4 ± 34.7	5.7 ± 1.5	14.5 ± 7.0	26.6 ± 18.6	144.7 ± 38.7
Parasympathetic ($N = 13$)	34.5 ± 13.9	64.1 ± 32.5	4.4 ± 2.1	11.1 ± 8.5	20.2 ± 10.5	116.4 ± 46.0
Combined ($N = 9$)	38.7 ± 13.1	129.3 ± 45.4	4.6 ± 1.3	14.0 ± 7.2	20.8 ± 9.8	131.2 ± 47.4

from cells only innervated by the parasympathetic system or whether different neural transmitters can cause differential secretion in the same cell. It is certain, however, that sympathetic stimulation does not elicit salivary flow by the expulsion of formed parasympathetic secretion.

The identification of protein material other than glycoprotein in cat submaxillary saliva is contrary to the report of Komarov and Stavraky (2), who, with the techniques available at that time, found that each type of saliva contained a single different glycoprotein and no other type of protein. The nature of the cat salivary proteins remains to be established.

The finding that cat submaxillary saliva produced by sympathetic nerve stimulation has higher [Na] and [K] than that evoked by parasympathetic nerve stimulation is in agreement with a previous report (10). In similar studies in the rat (11) and dog (12) only the [K] was found significantly higher.

It is well established that Na levels in parasympathetic saliva are a function of flow rate. In the present study it is clear that sympathetic stimulation produces a greater Na excretion at lower salivary flow rates than does parasympathetic stimulation. This indicates that the nature of the autonomic stimulus, as well as flow rate, must be considered when studying Na excretion. In relation to this, it is interesting to note that the combined stimulation of sympathetic and parasympathetic nerves produced [K] and [Ca] similar to those found in parasympathetic samples, whereas the combined [Na] was higher than in parasympathetic and closer to those sympathetic samples. In some cases the higher combined Na levels might be attributed to the fact that the combined flow rate was greater than that of the parasympathetic. However, there were four animals in which the parasympathetic flow rate was greater and the Na levels lower than those of the combined samples. This suggests that the sympathetic innervation to this gland may have a more important role in controlling Na than K or Ca excretion when both nerves are activated.

The higher [Ca] in the sympathetic secretion in this gland has not, to our knowledge, been previously reported.

Since salivary urea nitrogen concentration can be inversely related to flow rate (13) it is difficult, on the basis of the present data, to causally relate the higher urea nitrogen concentrations of the sympathetic samples to the nature of the stimulus, as these samples had the lowest rates of flow.

1. Burgen, A. S. V. and Emmelin, N. G., "Physiology of the Salivary Glands," Arnold, London (1961).

2. Komarov, S. A. and Stavraky, G. W., Can. J. Res., D. **18**, 222 (1940).

3. Davis, B. J., Ann. N. Y. Acad. Sci. **121**, 404 (1964).

4. Meyer, T. S. and Lamberts, B. L., Nature **205**, 1215 (1965).

5. Keyser, J. W., Anal. Biochem. **9**, 249 (1964).

6. Chaney, A. L. and Marbach, E. P., Clin. Chem. **8**, 130 (1962).

7. Kabat, E. A. and Mayers, M. M., "Experimental Immuno-chemistry." Thomas, Springfield, Illinois (1961).

8. Dische, Z., *in* "Methods of Biochemical Analysis" (D. Glick, ed.), Vol. 2, p. 237. Wiley (Interscience), New York (1955).

9. Walker, H. M. and Lev, J., "Statistical Inference." Holt, New York (1953).

10. Langstroth, G. O., McRae, D. R., and Stavraky, G. W., Arch. Intern. Pharmacodyn. **58**, 61

(1938).
11. Yoshida, Y., Sprecher, R. L., Schneyer, C. A., and Schneyer, L. H., Proc. Soc. Exptl. Biol. Med. **126**, 912 (1967).
12. Kesztyüs, L. and Martin, J., Arch. Ges. Physiol. **239**, 408 (1937).
13. Burgen, A. S. V., J. Cellular Comp. Physiol. **48**, 113 (1956).

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Effects of Local Alterations of pCO_2 and pH on Cerebrovascular Resistance in Isolated Dog Heads* (33546)

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The effect on cerebral vascular resistance of changing blood pCO_2 generally throughout the body has been studied extensively in animals and man using a variety of techniques (1–4). When pCO_2 is increased by adding CO_2 to the ventilating gas or by decreasing ventilation, cerebral vascular resistance falls and cerebral blood flow increases (1–4). While evidence indicates that the direct effect of CO_2 on cerebral blood vessels is dilation, there appear to be few definitive experiments showing the effect of locally altering pCO_2 and pH on cerebral vascular resistance. One author stated that the cerebral vasculature of the isolated cat head was largely unresponsive to local changes in pCO_2 and pH unless the alterations were extreme (5). However, this maneuver was a peripheral part of the study and no data were presented.

The present paper describes the effect of locally increasing and decreasing p_aCO_2 and pH by graded increments on the isolated cerebral circulation.

Methods. Adult mongrel dogs of both sexes were anesthetized intravenously with sodium thiopental (30 mg/kg) and heparinized (3 mg/kg).

Dog-pump-head. Each of five dog heads was perfused with blood from another dog (donor) as follows: A Sigmamotor pump was interposed between one carotid artery of the donor animal and two carotid arteries of the head of another dog. Blood returned by gravity from the cannulated jugular veins of the head to a jugular vein of the donor. The external carotid arteries were ligated, the head was severed from its body, and the spinal canal was plugged with bone wax. Wire ligatures prevented bleeding from the cut surfaces of the neck. Carotid artery pressure was measured distal to the perfusion pump with a Statham pressure transducer and recorded on a Schlumberger galvanometer recorder. The donor dog was ventilated with a positive pressure respirator using mixtures of percent CO_2 in O_2 over the range 0–14.5% CO_2. These mixtures were obtained continuously with an anesthetic gas machine. Approximately 10 min were allowed at each different concentration of CO_2. Percentage of CO_2 in the ventilating gas was monitored continuously with a Harvard critical orifice CO_2 analyzer. Cerebral arterial blood pH was measured at 5–10 min intervals with a Beckman model GS pH meter.

Cerebral blood flow was held constant in each experiment. Changes in cerebral artery perfusion pressure therefore indicated changes in cerebral vascular resistance in the same direction.

Oxygenator-pump-head. Six decapitate dog heads were perfused at constant flow with blood from an artificial oxygenator system. A diagram of the preparation is shown in Fig. 1. The system was primed with whole blood from the same dog and circulation was established through the dog's head via both caro-

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