PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 173, 467–470 (1983)

Salivary Secretion Induced by Substance P[1] (41671)

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Abstract. Secretion of fluid, ions, and amylase from parotid and submaxillary glands of rat, induced by intravenous injection of substance P (SP), was examined. The action of SP on salivary glands, like physalaemin, resembled that of cholinergic stimulation. While SP-evoked salivary flow from both glands was blocked by atropine, atropine did not modify composition of SP-evoked saliva. The present study suggests that salivary secretion and secretion of ions and amylase evoked by SP are mediated via SP-sensitive cholinergic receptors and specific SP receptors, respectively.

Substance P (SP), an undecapeptide that is a potent sialogogue, was originally found in the crude extract from bovine or rat hypothalamus by Leeman and Hammerschlag (1, 2). The mechanism of its sialogogic action or the composition of saliva produced by SP stimulation has been a subject of interest. Several *in vitro* studies showed that actions of SP on salivary glands were not blocked by autonomic antagonists (3). On the other hand, *in vivo* experiments by Inoki *et al.* (4) recently showed that SP-induced salivary flow was markedly reduced by atropine while SP-induced amylase secretion was markedly enhanced by α-adrenergic antagonists. On the basis of these findings, Inoki *et al.* (4) concluded that SP stimulated not only autonomic receptors but also SP specific receptors. More recently Martinez and his co-workers (5, 6) found that SP had both a direct secretory effect on the rat submaxillary gland and a modulating action on its response to acetylcholine and to isoproterenol. SP did not modify the binding of cholinergic or β-adrenergic receptors of rat submaxillary glands (6). Thus, the mechanism of action of SP remains to be explored.

Materials and Methods. Adult Long–Evans male rats (weighing 350–400 g) were maintained on standard lab chow and water *ad libitum* until 18 hr before experimentation when they were anesthetized by ip administration of pentobarbital sodium (50 mg/kg). Tracheotomy was performed to insure a clear airway. One side of the external jugular vein was exposed and a fine polyethylene cannula (Clay–Adams PE 50) was inserted to permit multiple infusions of SP and antagonists. For collection of submaxillary saliva, a fine polyethylene cannula (Clay–Adams PE 10) was inserted to a distance of 4 mm in the oral opening of one submaxillary duct, and saliva was collected from the end of the cannula using disposable micropipets (3 to 10 μl). Parotid saliva was collected directly from the cut end of the duct by micropipets. Rate of salivary flow was determined concurrently and related to the gland mass by obtaining the weight of the gland at the end of the experiments. Sample volumes of 10 and 3 μl were used for determination of salivary calcium and Na and K concentrations, respectively. Calcium concentrations of saliva and glandular samples were measured by titration of a calcein–calcium complex, using EGTA delivered by motorized calcette (Precision Systems) as described previously (7). Na and K concentrations of saliva were measured, after appropriate dilution of the 3-μl samples, by flame photometry using internal Li standard (Instrumentation Labs, Model 143). Amylase activity of saliva and glands was determined by the method of Myers *et al.* (8) as modified by Marsters *et al.* (9). SP was purchased from Beckman Instrument Company.

Results. A single dose of SP (5 μg/kg delivered in 40–60 sec) infused slowly into the jugular vein of rats elicited an immediate onset of salivary flow from both parotid and submaxillary glands. The flow, however, did not persist for more than a few minutes after cessation of the SP infusion. Initially (immedi-

[1] This work was supported by research Grants DE 05633 and DE 02110 from the National Institute of Dental Research, Bethesda, Maryland.

0037-9727/83 $1.50

ately after infusion of SP), flow rate from both glands was high; flow from both submaxillary and parotid declined sharply, ceasing within 5 min after injection with submaxillary and within 2–3 min with parotid. Salivary flow from both submaxillary and parotid glands did not vary appreciably with each successive infusion of SP in a particular experiment but there was some variability in flow rate from animal to animal.

The composition of SP-evoked secretions is shown by the data in Table I. Values presented in this table were mean concentrations of ions and activity of amylase of initial saliva samples collected following each SP infusion. Initial samples represent first samples collected after discarding first 5 μl of samples during each infusion of SP. There were usually 5–10 samples collected in quantities of 3–10 μl during each infusion of SP. SP evoked a parotid saliva with a characteristic ion and amylase pattern from all animals regardless of the absolute initial flow rate. The initial flow rate, even when low (18 μl/min/g) represented the high value for some animals. Thus, the [Na] of parotid saliva was uniformly high in these initial salivas, even though initial flow rate was quite variable from animal to animal; [K] and [Ca] and amylase activity of initial salivas were similar for all animals (Table I). The [Na] of submaxillary saliva evoked immediately following infusion of SP was 52 ± 7 (eight rats) despite the wide variation in initial flow rate. With submaxillary, as with parotid, the flow rate initially represented the "high" value for any particular animal. There was, however, a time-related change in flow rate and [Na]. The [K] and [Ca] of initial submaxillary saliva samples were similar for all animals (Table I). No amylase activity was found in any SP-evoked submaxillary saliva.

Glandular [Ca] of both submaxillary and parotid glands was not changed following 60 min of stimulation with SP; amylase activity of parotid was also unchanged (Table II).

SP-induced salivary flow from both glands was markedly decreased (50–90%) following intravenous injections of atropine (5–100 μg/kg). This inhibition was reversible, since 70–90 min after the administration of atropine, the infusion of SP elicited a flow rate similar to that obtained prior to atropine treatment. The atropine-induced inhibition was also overcome by doubling SP doses. However, atropine (5–100 μg/kg, iv) did not significantly modify ion concentrations of SP-evoked saliva obtained from either gland. To show effects of atropine on [Na] and flow rate of SP evoked saliva, it is necessary to present data from individual animals since there is the wide range in absolute values of the initial salivas. Thus, [Na] of SP-evoked parotid saliva was initially 129 meq/liter, at a flow rate of 79 μl/min/g; [Na] of saliva evoked by SP 10 min after iv infusion of atropine was 133 meq/liter but flow rate was reduced to 30 μl/min/g representing a decrease of 62% in flow rate but no significant change in [Na]. This same pattern (but not absolute values) was obtained for three additional rats. Similar relations existed

TABLE I. FLOW RATE AND COMPOSITION OF SUBSTANCE P-EVOKED RAT SALIVA

Initial flow rate (μl/min/g)	Concentration			
	Na (meq/liter)	K (meq/liter)	Ca (meq/liter)	Amylase (mg/mg)
		Parotid saliva		
18–77	135 ± 3 (18)	28 ± 2 (17)	8.5 ± 0.5 (9)	46 ± 10 (9)
		Submaxillary saliva		
66–274	52 ± 7 (8)	53 ± 4 (8)	0.9 ± 0.1 (15)	0

Note. Values are means ± SEM. Substance P (5 μg/kg) was administered intravenously via jugular vein every 5–10 min for 5 doses. Amylase is expressed as number of milligrams of reducing substance as glucose, formed in a 15-min digestion period at 37°C per milligram of saliva. The Na, K, and Ca are expressed as meq/liter of saliva. Flow rate is expressed as microliters per minute per gram of gland weight.

TABLE II. EFFECT OF SUBSTANCE P ON GLANDULAR AMYLASE AND [Ca] OF SALIVARY GLANDS

	Glandular amylase (mg/mg wet wt)	Glandular [Ca] (meq/kg wet wt)	
	Parotid	Parotid	Submaxillary
Before SP	722 ± 103	10.6 ± 1.1	10.8 ± 1.0
After SP	712 ± 85	10.4 ± 0.8	10.4 ± 1.5

Note. Values are means ± SE of four experiments. SP was infused (5 μg/kg, iv) at 5- to 10-min intervals over a period of 60 min. Amylase activity is expressed as milligrams reducing substance producing a 15-min digestion per milligram fresh wet weight.

with submaxillary. The [Na] of SP-evoked submaxillary saliva was initially 59 meq/liter and flow rate was 121 μl/min/g; after atropine, [Na] of SP-evoked saliva was 61 meq/liter and flow rate was 14.1 μl/min/g, again flow rate was decreased (in this case 88%) from preatropine values, while [Na] was unaffected. The [K] and [Ca] for parotid saliva following atropine treatment in four experiments were 28 ± 4 and 9.5 ± 0.3, respectively, and for submaxillary saliva, 45 ± 2 and 1.1 ± 0.1 meq/liter, respectively. Amylase activity of SP-induced parotid saliva (50 ± 2 mg/mg) was also not altered by atropine.

Discussion. The present study provided the first report of composition of SP-evoked parotid secretion. The composition of SP-evoked parotid as well as submaxillary saliva is very similar to that evoked by physalaemin (12) and also resembles cholinergically evoked saliva for most parameters measured. It is difficult to correlate changes in [Na] and flow rate under the particular conditions of these experiments. With submaxillary, there did appear to be a time-related decrease in [Na] and flow rate when successive samples of saliva were obtained from any particular rat. The decrease in flow rate could be the result of falling blood levels of SP. Martinez also showed a decrease in [Na] and flow rate of submaxillary saliva during constant infusion of a particular dose of SP (6). It thus appears that the decreases observed could most properly be interpreted as time-related changes in the tissue response since there was no change in dose of the drug. Similarly, our data may be interpreted as time-related changes in response to the tissue, although it is possible that the change in blood levels of SP were solely responsible for the diminishing flow rate.

The [Na] of SP-evoked or physalaemin-evoked submaxillary saliva was somewhat higher than that of cholinergically evoked saliva (11). Recently, Coreneo *et al.* (13) reported that physalaemin-evoked saliva was poorer in both Na and K at all flow rates. They concluded that, in contrast to acetylcholine and carbamylcholine, which are known to inhibit Na reabsorption and stimulate K secretion in the isolated perfused rat submaxillary duct, physalaemin had the opposite effects (13). The higher [Na] in SP-evoked submaxillary saliva observed in the present study suggests that SP might act directly on ductal transport of this ion. Moreover, Martinez *et al.* have reported that SP acts directly not only on acinar cells but also on duct cells of submaxillary glands (5, 6).

The present study shows that atropine, a known cholinergic antagonist, at doses used to inhibit flow rate and modify salivary composition of cholinergically evoked saliva (10), blocked salivary flow of both SP-evoked parotid and submaxillary saliva without altering their compositions. On the basis of the present data, it may be concluded that cholinergic receptors as well as SP-receptors are activated by SP. Existence of specific SP-receptors in salivary glands (14) had in fact been demonstrated. However, it is not known what role specific SP receptors play in SP-induced salivary secretion, and demonstration of the role of SP-receptors must await availability of a specific SP-receptor antagonist.

1. Hammerschlag R, Leeman SE. Induction of salivation by a bovine hypothalamic factor. Fed Proc **25**:192, 1966.
2. Leeman SE, Hammerschlag R. Stimulation of salivary secretion by a factor extracted from hypothalamic tissue. Endocrinology **81**:803–810, 1967.
3. Rudich L, Butcher FR. Effect of Substance P and eledoisin of K^+ efflux, amylase release and cylcic nucleotide levels in slices of rat parotid gland. Biochim Biophys Acta **444**:704–711, 1976.
4. Inoki R, Jido T, Kotani Y, Oka M, Iwatsubo K, Matsumoto K. Mechanism of substance P-induced salivary secretion in rats. Advan Exp Med Biol **120A**:409–420, 1979.
5. Martinez JR, Martinez AM. Direct and modulating secretory substance P on rat submandibular gland. Advan Physiol Sci **28**:53–58, 1981.

6. Martinez JR, Martinez AM. Stimulatory and inhibitory effects of substance P on rat submandibular secretion. J Dent Res **60**:1031–1038, 1981.
7. Yu JH, Schneyer CA. Parasympathetically-evoked parotid salivary secretion of chronically amitriptyline-treated rats. Experientia **38**:945–946, 1982.
8. Myers AC, Free AH, Rosinski EE. Studies of animal diastases. VI. The determination of diastase (amylase) in blood. J Biol Chem **154**:39–48, 1944.
9. Marsters RW, Kinney TD, Linn KY. A micromethod for the determination of plasma amylase. Clin Chem **6**:130–139, 1960.
10. Schneyer CA, Hall HD. Comparison of rat saliva evoked by auriculo-temporal and pilocarpine stimulation. Amer J Physiol **209**:484–488, 1965.
11. Schneyer CA, Schneyer LH. Electrolyte levels of rat salivary secretions. Proc Soc Exp Biol Med **101**:568–569, 1959.
12. Schneyer CA, Hall HD. Characterization of physalaemin-evoked rat saliva and failure of autonomic blocking agents to modify composition. Proc Soc Exp Biol Med **127**:1245–1248, 1968.
13. Coroneo MT, Denniss AR, Young JA. The action of physalaemin on electrolyte excretion by the mandibular and sublingual salivary glands of the rat. Pfluegers Arch Europ J Physiol **381**:223–230, 1979.
14. Putney JW Jr, Vandewalle CM, Wheeler CS. Binding of ^{125}I-physalaemin to rat parotid acinar cells. J Physiol (London) **301**:205–212, 1980.

Received October 21, 1982. P.S.E.B.M. 1983, Vol. 173.