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Salivary Secretion of a Substance Immunologically Identical to the α-Subunit of 7S Nerve Growth Factor[1] (40201)

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7S Nerve growth factor (7S NGF) is present in an exceedingly high concentration in the submaxillary glands of male mice (1, 2). This protein has a molecular weight around 140,000 and contains α-, β-, γ-subunits (2, 3). Both 7S NGF and its β-subunit (β-NGF) can stimulate the outgrowth of nerve fibers from sensory and sympathetic ganglia (2). In contrast, neither the α-subunit nor the γ-subunit can promote such nerve fiber outgrowth. The α-subunit of 7S NGF has been reported to have a protective effect on sensory ganglion cells during trypsin dissociation (4) and the γ-subunit has been shown to possess esteroproteolytic activity (5).

The relationship between this large pool of NGF proteins and the physiological functions of the salivary glands is not yet clear. It has been suggested that the submaxillary gland might function as both an endocrine (6) and exocrine (7–9) organ for NGF. An early study by Levi-Montalcini and Cohen (7) reported that pilocarpine-induced mouse saliva contained very low but detectable levels of biologically active NGF. Subsequent experiments in this laboratory have shown that mouse saliva elicited by certain sympathomimetic agonists contains an exceedingly high concentration of a substance which stimulates nerve fiber outgrowth from sympathetic ganglia and is immunologically similar to β-NGF (8). The concentration of β-NGF as estimated by bioassay was much higher in saliva elicited by α-adrenergic agonists than in that elicited by either β-adrenergic or muscarinic agonists (8). High concentrations of immunoreactive β-NGF have also been reported by Murphy *et al.* (9) in control, unstimulated mouse saliva. These observations strongly suggest that the mouse submaxillary gland serves as an exocrine organ for β-NGF. In contrast, studies of the possibility that the submaxillary gland might also serve as an endocrine organ for NGF are equivocal. Thus, Hendry and Iverson (6) reported that sialectomy resulted in a profound fall in the plasma level of β-NGF while similar experiments by Murphy *et al.* (9) did not result in a decreased plasma level of β-NGF.

The results reported in the present paper demonstrate that mouse salivary secretions contain a substance which is immunologically identical to the α-subunit of 7S NGF. In addition, data are presented which show that α-subunitlike material is preferentially released into the saliva by activation of the α-adrenergic receptors within the salivary glands. These results parallel those previously obtained for β-NGF (8) and strongly suggest that the mouse salivary glands serve as exocrine organs for both the α- and β-subunits of 7S NGF.

Materials and methods. Saliva was elicited from 10- to 16-week-old male mice. The animals were anesthetized with pentobarbital, 60 mg/kg. Salivation was induced by the intraperitoneal injection of a secretagogue. The secretagogues and ranges of doses for each were: pilocarpine, 0.1–0.8 mg/kg; epinephrine, 2.0–6.6 mg/kg; norepinephrine, 0.7–2.5 mg/kg; and isoproterenol, 1.0–5.0 mg/kg. In a few cases, about one-tenth the intraperitoneal dose of secretagogue was injected under the sheath of connective tissue covering the submaxillary gland. The results obtained with locally administered epinephrine and pilocarpine were comparable to those after intraperitoneal injection of these secretagogues. In one series of animals, phenoxybenzamine HCl (5 mg/kg; Smith, Kline, and French, Philadelphia, PA) was administered by slow injection into the jugular vein 30–60 min prior to intraperitoneal injection of the secretagogue.

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Saliva was collected in a microcapillary tube placed between the tongue and the floor of the mouth. (The ducts from the submaxillary and sublingual glands open into the buccal cavity under the tongue). In some experiments, small aliquots of saliva were collected sequentially from a single animal given one dose of a secretagogue. In other cases, all of the saliva elicited by one dose of a secretagogue was collected as one sample. Immediately after collection, the saliva was frozen at −40° until the assays were performed.

Complement fixation was carried out following the method of Moore *et al.* (10). Samples of saliva or of purified proteins were diluted in sodium veronal buffer (5 m*M*, pH 7.5; Ref. 11) containing 0.1% (w/v) gelatin and 0.005% (v/v) Triton X-100. A 5 μl aliquot of each dilution was added to a 50 μl mixture consisting of complement-containing guinea pig serum and horse antiserum produced against the β-subunit of nerve growth factor (Burroughs-Wellcome Co., Research Triangle Park, NC) at a final dilution of 1:350 and 1:555, respectively, in the same buffer. The resultant mixture was incubated at 4° for 18–24 hr. After this incubation, 0.3 ml of a suspension of sheep red blood cells sensitized by treatment with glycerinated antisheep hemolysin (Difco, Detroit, Mich; final dilution 1/300, v/v) were added to each reaction mixture. The samples were incubated for 60 min at 37° to allow complement-mediated hemolysis and then centrifuged to pellet the red blood cells (12,000*g* for 10 min). The absorbance of the supernatant at 413 nm was determined. The optical density of the blanks which had 5 μl of buffer added in place of the sample was usually close to 1.0. (This parameter was initially set by adjusting the amount of complement in the reaction.) All samples were assayed at several dilutions. The reciprocal of the dilution which yielded 50% of maximum complement fixation is defined as the number of complement fixation units (CF_{50}) in 5 μl of undiluted sample. The results are expressed in relative concentrations (i.e., CF_{50}/μg protein) because of variability in the CF_{50} value of the purified α-subunit (see Results).

In all experiments, controls lacking antiserum to nerve growth factor were used to assess the anticomplementary activity of all unknowns. While the standards did not exhibit anticomplementary activity, the saliva samples showed 50% hemolysis due to anticomplementary activity at a concentration 10-fold greater than the concentration needed for 50% complement fixation (12).

In studies involving inhibition of complement fixation by antigen excess, a 5 μl aliquot containing 300 ng of either the α-subunit or β-subunit of 7S NGF was added to the reaction mixture in addition to the 5 μl of diluted sample. The remainder of the procedure was the same as that described above.

Immunodiffusion was carried out as follows. 1.5% agarose (Bio-Rad Laboratories, Richmond, CA) was prepared in phosphate-buffered saline (13) with 0.1% (w/v) sodium azide and plated in 50 × 12 mm Falcon dishes having tight-fitting lids (Oxnard, CA). Ten microliter aliquots of solution containing antigen or antibody were added to each well and the dishes were incubated for up to 72 hr at room temperature. Dishes were stained with Coomassie blue as described by Ouchterlony and Nilsson (13).

Protein was determined by the Lowry method (14) with bovine serum albumin (fraction V, 96–99% albumin; Sigma Chemical Co., St. Louis, MO) as a standard. The concentration of the standard protein solution was calculated by use of the extinction coefficient of albumin at 280 nm ($E^{1\%}_{1cm} = 6.6$; Ref. 15).

Samples of purified α-, β-, or γ-subunit of 7S NGF and intact 7S NGF were generously provided by Dr. Eric Shooter (Depts. of Genetics and Biochemistry, Stanford University). Mouse gamma globulins (fraction II) were purchased from Miles Laboratories, Inc., Kankakee, IL. In most experiments, these proteins were dissolved in cold (0°) sodium veronal buffer containing both gelatin and Triton X-100 (see above).

Results. Complement fixation was used to determine whether samples of mouse saliva contain antigens capable of binding antibodies present in the Burroughs-Wellcome antiserum against the β-subunit of 7S NGF. It was found that salivary secretions elicited by a variety of secretagogues all contain such antigens. Furthermore, the concentrations of antigens capable of binding the antibodies in

the NGF antiserum varied markedly in secretions elicited by different autonomic agonists (Fig. 1). These results show that aliquots of mouse saliva elicited by epinephrine, norepinephrine, and isoproterenol, respectively, contained 1,600, 370, and 33 times as much NGF-like immunoreactive protein as that elicited by the muscarinic agonist pilocarpine.

Preparations of purified antigens were tested for activity in the complement fixation assay in an attempt to characterize the salivary antigens which bind the antibodies in the Burroughs-Wellcome nerve growth factor antiserum. Complement fixation curves using Burroughs-Wellcome antiserum are shown in Fig. 2 for each subunit of 7S NGF and for mouse gamma globulins, which contaminate preparations of β-NGF (16). These results demonstrate that the antiserum contains antibodies against all three subunits and mouse gamma globulins. When solutions containing these antigens are prepared as described in Materials and methods, the CF_{50} values for the α-, β-, and γ-subunits, and gamma globulins are 1.6 ± 0.3 (n = 8), 1.4 ± 0.1 (n = 8), 52 ± 4 (n = 7), and 0.59 ± 0.04 (n = 4) ng of protein, respectively. These CF_{50} values varied with small changes in the way in which the standard solutions were prepared. For example, preincubation of the standard solutions for 1 hour at 0° lowered the CF_{50}

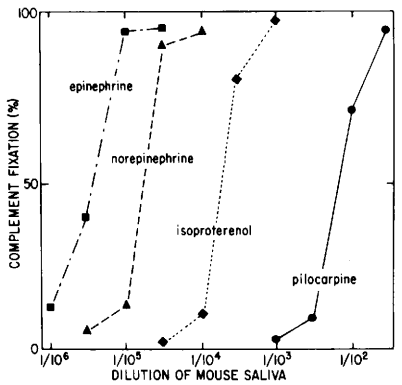

FIG. 1. Complement fixation analysis of mouse saliva elicited by four different autonomic secretagogues. The relative concentration of immunoreactive NGF-like protein in each saliva is proportional to the reciprocal of the dilution at which the complement fixation curve crosses 50% (i.e., CF_{50}). The aliquots of saliva used in this experiment were obtained from four separate mice and were assayed using the Burroughs-Wellcome antiserum to β-NGF as described in Materials and methods.

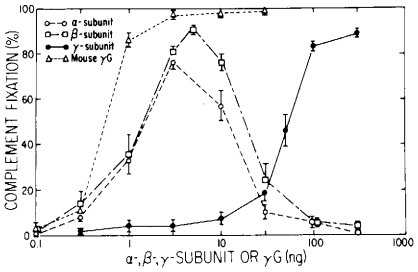

FIG. 2. Complement fixation analysis of the α-, β-, and γ-subunits of 7S nerve growth factor and of mouse gamma globulins using the Burroughs-Wellcome antiserum to β-NGF. Purified proteins were obtained and assayed as described in Materials and methods.

values for both the α- and β- subunits to ~0.5 ng of protein (control curves, Fig. 3). CF_{50} values as low as 0.14 ng protein have been observed for some preparations of the α-subunit. This variability in activity in the complement fixation assay might reflect differences in the extent of solubilization and/or the molecular configuration of the antigens.

The complement fixation curves shown in Figs. 2 and 3 demonstrate decreasing complement fixation in the region of antigen excess for both the α- and β-subunits of 7S NGF. Such inhibition by antigen excess is a normal feature of the complement fixation technique (17). It was impossible to determine whether the γ-subunit also showed inhibition by antigen excess because of its poor reactivity (Fig. 2). In contrast, excess amounts of the mouse gamma globulins did not appear to inhibit complement fixation (Fig. 2). This result is probably explained by the presence of multiple immunoreactive proteins in this complex blood fraction whose individual complement fixation curves would sum to yield a composite complement fixation curve (*vide infra*).

Addition of an inhibitory amount of the α-subunit (300 ng/tube) completely blocked complement fixation by additional α-subunit but had no effect on complement fixation by β-NGF, the γ-subunit, or gamma globulin (Figs. 3 and 4). Similarly, excess β-NGF only inhibited complement fixation by β-NGF (Figs. 3 and 4).

Application of this inhibition test to the composite complement fixation curve obtained with epinephrine-induced mouse saliva demonstrated that the salivary antigen

quantified by complement fixation is immunologically similar to the α-subunit of 7S NGF. Addition of an inhibitory amount of the α-subunit shifted the complement fixation curve by approximately fourfold (Fig. 5). In contrast, addition of an inhibitory amount of β-NGF had no effect on the salivary complement fixation curve (Fig. 5). Similar results were obtained using saliva elicited by norepinephrine, isoproterenol, and pilocarpine.

Examination by immunodiffusion of mouse saliva elicited by epinephrine revealed

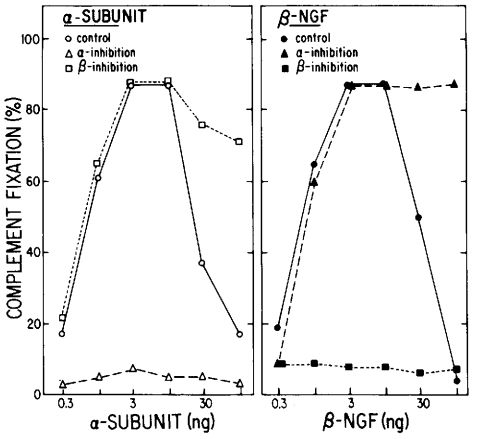

FIG. 3. Complement fixation by the α- and β-subunits of 7S NGF incubated in the presence or absence of excess amounts of either the α- or β-subunit. Curves labeled "α-inhibition" or "β-inhibition" were obtained by addition of 300 ng/tube of either α- or β-subunit, respectively. Complement fixation was carried out as described in Materials and methods. Standards were held on ice for 1 hr before use.

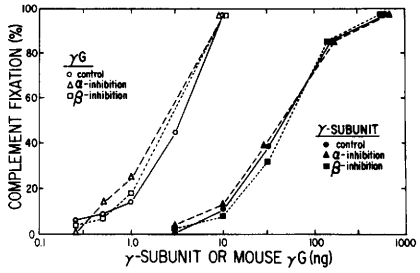

FIG. 4. Complement fixation by the γ-subunit of 7S NGF and mouse gamma globulins incubated in the presence or absence of excess amounts of either the α- or β-subunit. Curves labeled "α-inhibition" or "β-inhibition" were obtained by addition of 300 ng/tube of either α- or β-subunit, respectively. Complement fixation was carried out as described in Materials and methods. Standards were held on ice for 1 hr before use.

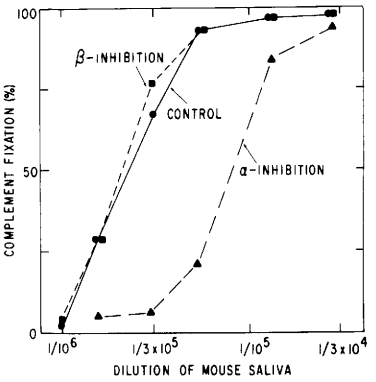

FIG. 5. Complement fixation by epinephrine-induced mouse saliva incubated in the presence or absence of excess amounts of the α- or β-subunit of 7S NGF. Curves labeled "α-inhibition" or "β-inhibition" were obtained by addition of 300 ng/tube of either the α- or β-subunit, respectively. Complement fixation was carried out as described in Materials and methods.

three immunoprecipitin bands which individually showed immunological identity with the α-subunit, β-NGF, or one of the mouse gamma globulins (Fig. 6).[2] The immunoprecipitin band corresponding to the α-subunit of 7S NGF could only be detected by concentrating the antiserum by 7.5-fold and diluting the saliva by 10- to 150-fold (Fig. 6B). In contrast, the immunoprecipitin bands corresponding to β-NGF (inner band, Fig. 6A) and mouse gamma globulins[3] (outer band, Fig. 6A) could best be visualized by concentrating the mouse saliva by 5-fold and using the stock concentration of antiserum. Since precipitin bands form where antigens and antibodies meet in equivalent proportions, these results suggest that (a) the saliva might contain more of the α-subunit than β-NGF or mouse gamma globuins, and/or (b) the antiserum might contain lower titers of antibody directed against the α-subunit than against β-NGF or mouse gamma globulins. As a result, the α-subunit of 7S NGF should

[2] No immunoprecipitin band was observed under any condition which might correspond to the poorly reactive γ-subunit of 7S NGF.

[3] The immunodiffusion pattern for the mouse gamma globulins demonstrates the presence of multiple types of immunoreactive proteins in this blood fraction. The outer immunoprecipitin band in the mouse saliva appears to show complete immunological identity with one of these proteins.

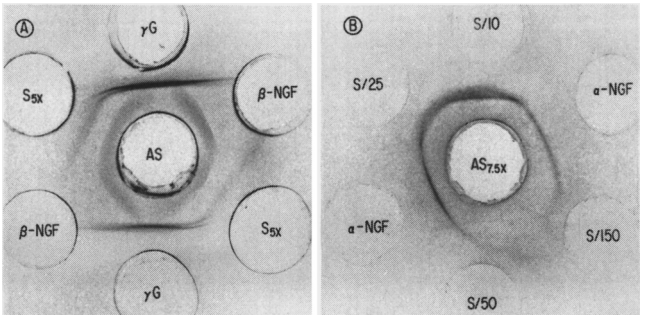

FIG. 6. Immunodiffusion analysis of epinephrine-induced mouse saliva. (A) Mouse saliva was concentrated fivefold (S_{5x}) in order to demonstrate the β-NGF and γ-globulin (γG) immunoprecipitin bands. Each β-NGF well contained 5 μg protein. The upper γG well contained 10 μg protein while the lower well contained 5 μg. (B) The antiserum was concentrated 7.5-fold ($AS_{7.5x}$) and the saliva was diluted from 10-fold (S/10) to 150-fold (S/150) in order to demonstrate the α-subunit immunoprecipitin band. Each α-subunit (α-NGF) well contained 4 μg protein. Immunodiffusion was carried out as described in Materials and methods using the Burroughs-Wellcome antiserum to β-NGF.

be detectable by complement fixation at higher dilutions of mouse saliva than either β-NGF or the mouse gamma globulins. This conclusion is consonant with the results presented in the preceding paragraph which indicated that the salivary antigen quantified by complement fixation is probably the α-subunit. The substance in saliva which is immunologically identical to the α-subunit of 7S NGF will be referred to as α-subunitlike material.

As a result of the above considerations, complement fixation was used to determine the relative concentrations of α-subunitlike material in sequential aliquots of mouse saliva collected after a single injection of either epinephrine or pilocarpine (Fig. 7). When epinephrine rather than pilocarpine was used as the secretagogue, the results showed several marked contrasts: (a) the total volume collected was much smaller (107 ± 14 μl (n = 3) vs. 410 ± 150 μl (n = 3)), (b) the duration of salivation was longer (Fig. 7), (c) the protein concentrations were 50-fold greater (Table I), and (d) the relative concentrations of the α-subunitlike material were 1000-fold greater (Fig. 1).

The relationship between protein concentration and relative concentration of α-subunitlike material was examined in mouse salivas elicited by norepinephrine and isoproterenol in order to determine whether the epinephrine-induced secretion of the α-subunit was due to activation of α-adrenergic or β-adrenergic receptors. These data along with

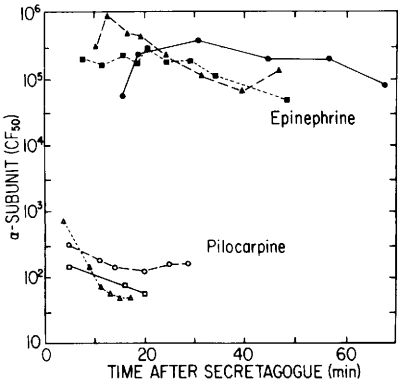

FIG. 7. Relative concentrations of a substance immunologically similar to the α-subunit of 7S NGF in sequential samples of saliva elicited after a single injection of epinephrine or pilocarpine. Each set of like symbols represents data from a single animal. Aliquots were collected every few minutes until salivation ceased; thus, the duration of salivation following epinephrine (filled symbols) was roughly twice that following pilocarpine (open symbols). Saliva samples were collected and assayed as described in Materials and methods. Data on the concentration of α-subunit-like material are given as CF_{50} units in the entire 5 μl aliquots assayed by complment fixation. The ordinate scale is logarithmic with index marks at 10, 30, 100, etc.

the corresponding data for epinephrine treatment are shown in Fig. 8. The relative specific activities (i.e., slopes of the curves) were determined by linear regression. The results shown in Table I demonstrate that the relative specific activity of the α-subunitlike material is more than 25-fold greater in saliva elicited by secretagogues having α-adrenergic

TABLE I. PROTEIN CONTENT AND RELATIVE SPECIFIC ACTIVITY OF A SUBSTANCE IMMUNOLOGICALLY SIMILAR TO THE α-SUBUNIT OF 7S NGF IN SECRETIONS ELICITED BY VARIOUS AUTONOMIC AGENTS.[a]

Autonomic agonist	Protein (μg/ml)	α-Subunitlike material (CF_{50}/μg protein)
Epinephrine (24) [α + β-adrenergic]	74 ± 9	790 ± 85
Norepinephrine (8) [α-adrenergic]	19 ± 5	810 ± 220
Isoproterenol (8) [β-adrenergic]	28 ± 2	33 ± 6
Pilocarpine (23) [cholinergic]	1.4 ± 0.2	29 ± 5

[a] Specific activities are the slopes of linear regression lines ± the SE of the slope. The regression lines for all data groups except pilocarpine are shown in Fig. 8. Numbers of samples are shown in parentheses. The receptors primarily stimulated by each agonist are indicated in square brackets. The following specific activities are statistically significant ($P < 0.01$): (a) pilocarpine vs both epinephrine and norepinephrine, and (b) isoproterenol vs both epinephrine and norepinephrine. Protein values are means ± SE.

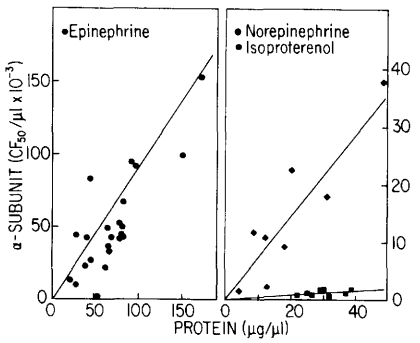

FIG. 8. Correlation between levels of α-subunitlike material and protein in secretions elicited by adrenergic agents. The lines were determined by linear regression analysis. The correlation coefficients for epinephrine, norepinephrine and isoproterenol were 0.86, 0.89, and 0.13, respectively. All values are given per μl of undiluted mouse saliva.

agonist activity than in that elicited by either the β-adrenergic agonist isoproterenol or the muscarinic agonist pilocarpine. These data suggest that α-subunitlike material is secreted as a consequence of the action of adrenergic agonists on α-receptors.

Prior administration of phenoxybenzamine, a selective blocker of α-adrenergic receptors, has previously been demonstrated to almost completely inhibit salivation following either norepinephrine or epinephrine (8). In a series of five phenoxybenzamine-treated animals, administration of epinephrine only yielded one sample of saliva of sufficient size (10 μl) to permit determination of both the concentration of protein and of the α-subunit. The relative specific activity of this sample (36 CF_{50}/μg of protein) was equivalent to that obtained after administration of the β-adrenergic agonist isoproterenol but far less than that elicited after the α-adrenergic agonist norepinephrine (Table I).

Discussion. The results presented in this paper demonstrate that substances immunologically identical to the α- and β-subunits of 7S NGF are present in mouse saliva elicited by either adrenergic or cholinergic agonists. Immunodiffusion analysis reveals that epinephrine-induced mouse saliva contains substances which show complete immunological identity with the single immunoprecipitin bands formed by purified β-NGF (Fig. 6A) and the α-subunit of 7S NGF (Fig. 6B). In addition, inhibition of complement fixation by mouse saliva incubated in the presence of large amounts of the α-subunit demonstrates the presence of a salivary antigen immunologically similar to that subunit (Fig. 5).

Data gathered on mouse saliva elicited by a variety of autonomic agonists demonstrate that α-subunitlike material is preferentially released by activation of α-adrenergic receptors. The relative specific activity of saliva elicited by agonists possessing α-adrenergic activity (epinephrine and norepinephrine) was more than 25-fold greater than that in saliva elicited by either a β-adrenergic or muscarinic agonist (isoproterenol and pilocarpine, respectively; Table I). Previous studies in this laboratory with receptor blockers (atropine, propranolol, and phenoxybenzamine) demonstrated that both epinephrine and norepinephrine elicit salivation by a highly selective activation of α-adrenergic receptors, while isoproterenol and pilocarpine elicit salivation by selective activation of β-adrenergic and muscarinic receptors, respectively (8). The relative specific activity of α-subunitlike material in a small sample of epinephrine-induced saliva obtained from a phenoxybenzamine-treated animal demonstrates that little of this material can be released when the α-adrenergic receptors are

blocked. These results support our conclusion that α-subunitlike material is secreted by activation of α-adrenergic receptors.

Previous studies in this laboratory have shown that, as with the α-subunitlike material, both β-NGF (8) and an anticomplementary factor (12) are preferentially secreted in mouse saliva by activation of α-adrenergic receptors. No basal salivary secretion was observed in either the present paper or in two previous studies (8, 12) in which mice were anesthetized with pentobarbital. In contrast, Murphy *et al.* (9) observed high levels of β-NGF in resting salivary secretions obtained from mice treated with the basal anesthetic tribromoethanol. Such resting salivary secretion is much more likely in animals treated with a light basal anesthetic than in animals anesthetized with a barbiturate. For example, Emmelin *et al.* (18) reported that pentobarbital blocked the resting secretion from the submaxillary glands of rats initially anesthetized with the basal anesthetic chloralose. Thus, the absence of resting salivary secretions in our studies and the presence of such secretions in the study by Murphy *et al.* (9) is probably due to the difference in the anesthetic. The basal salivary secretion observed by Murphy *et al.* (9) could have resulted from (a) spontaneous activity on the part of certain salivary gland cells (i.e., spontaneous secretion; ref. 19) (b) sympathetic hyperactivity caused by operative trauma (18), irritation due to administration of the anesthetic tribomoethanol solution[4] (18), or reflex mechanisms activated by the hypotension and respiratory depression caused by tribromoethanol (20) or (c) the normal reflex mechanisms associated with regulating salivary secretion in the intact animal (21, 22). Thus, high levels of β-NGF might be present in such resting salivary secretions if they resulted from the activation of the sympathetic input to the submaxillary gland.

Application of the complement fixation inhibition test to highly purified samples of the α-, and β-, and γ-subunits of 7S NGF suggests that the subunits do not share common antigenic determinants. Thus, complete inhibition of complement fixation by the α-subunit had no effect on complement fixation by either the β- or γ-subunit (Figs. 3 and 4). Similarly, complete inhibition of complement fixation by β-NGF had no effect on complement fixation by either the α- or γ-subunit (Figs. 3 and 4). In contrast, Tomita and Varon (16) reported that the α- and γ-subunits share some, but not all, antigenic determinants.

The complement fixation inhibition test also demonstrated that the immunoreactivity of the gamma globulin preparation cannot be explained by the presence of contaminating α- and β-subunits (Fig. 4). Contamination by the γ-subunit is not a possible explanation as the purified γ-subunit is 100-fold less immunoreactive than gamma globulin (Fig. 2). Thus, it appears that specific antibodies to one or more of the gamma globulins are present in the Burroughs-Wellcome antiserum to nerve growth factor.

These results demonstrate that the commercial antiserum prepared against purified β-NGF contains separate pools of antibodies directed against each of the subunits of 7S NGF and also against mouse gamma globulins. Thus, the β-NGF prepared by Burroughs-Wellcome by the method of Varon, Nomura, and Shooter (23) must have contained trace amounts of the α-subunit, the γ-subunit, and one or more of the mouse gamma globulins. This confirms earlier observations by Tomita and Varon (16) who reported that β-NGF contained trace amounts of gamma globulin. The presence of antibodies to all of the subunits of 7S NGF and to mouse gamma globulins must be taken into consideration when evaluating experiments employing the Burroughs-Wellcome antiserum. For example, it seems possible that some of the complex anatomical, biochemical, and physiological effects (24) seen subsequent to injection of antiserum to NGF in order to achieve immunosympathectomy might result from the presence of antibodies against the α- or γ-subunits of the 7S NGF. In support of this idea, Varon and Raiborn (4) have shown that the α-subunit is in some way able to "protect" sensory neurons and glia during cell dissociation. In addition, unpurified Burroughs-Wellcome antiserum should never be employed in immunofluorescent studies of β-NGF localization.

Summary. Mouse saliva elicited by several

[4] The camphoraceous odor and burning taste associated with the solution of tribromoethanol in t-amyl alcohol suggest that the solution might well have local irritant properties (20).

secretagogues is shown by immunodiffusion and complement fixation to contain a factor immunologically identical to the α-subunit of 7S nerve growth factor. The relative specific activities of α-subunitlike material are 790, 810, 33, and 29 complement fixation units per μg of protein in secretions elicited by epinephrine, norepinephrine, isoproterenol, and pilocarpine, respectively. Thus, samples of saliva elicited by agonists which activate α-adrenergic receptors have a 25-fold greater relative specific activity than those elicited by agonists which activate either β-adrenergic or muscarinic receptors. In light of earlier experiments on the effects of specific receptor blockers on drug-induced salivation, these results strongly suggest that α-subunitlike material is preferentially released by activation of α-adrenergic receptors. In addition, data are presented which suggest that the α-, β-, and γ-subunits of 7S nerve growth factor do not have common antigenic determinants. Finally, Burroughs-Wellcome antiserum prepared against the β-subunit of nerve growth factor is shown to contain antibodies against all three subunits and one or more of the mouse gamma globulins.

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