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EVIDENCE FOR THE RELEASE AND POSSIBLE NEURAL REGULATION OF NITRIC OXIDE IN HUMAN SALIVA

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We report the presence of nitric oxide (NO) as measured through its product NO_2 in human saliva. The presence of NO is not due to oral bacterial activity but is due to a genuine release of NO from the salivary glands and can be detected in freshly secreted saliva collected from a mouth freshly rinsed with an antiseptic preparation to reduce the bacterial content significantly. In order to study the regulation of salivary NO we used lemon juice to increase salivary flow and detected an immediate transient reduction of salivary NO_2 which rose to levels equal to and sometimes higher than basal levels in five minutes in most donors indicating salivary NO production might be under neural control. Salivary NO production may play a physiological role in both the natural antibacterial properties of salivary secretion and possibly in detoxification of oral carcinogens.

NO is a noxious, and free radical gas which has recently been shown to have manifold functions including regulation of immune function by mediating tumoricidal and bactericidal functions in macrophages, blood vessel dilatation by accounting for the activities of endothelium derived relaxing factor, and neurotransmission in the central and peripheral peripheral nervous systems (1). NO is synthesized from L-arginine by NO synthase (NOS) which is made up of at least two isoforms (2): a constitutively expressed isoform which is Ca^{+2} and calmodulin dependent, and is expressed in some tissues of the body notably endothelium, neurons, and platelets; and an inducible isoform which is Ca^{+2} and calmodulin independent, and when induced can trigger production of relatively large amounts of NO in macrophages, hepatocytes, and many other cell types. Recent studies indicate both the inducible and constitutive isoforms of NOS not to be confined to separate cell types, but to exist in the same cell (1).

The secretion of NO in saliva has so far not been reported. The salivary gland secretions contain in addition to water, electrolytes, digestive enzymes, proteins, and antibodies which are central to the delivery of digested meal to the small intestine as well as for cleansing the mouth and teeth to prevent disease such as dental caries. We report here that freshly released human saliva contains measurable and sometimes relatively high levels of NO and that the output of NO can be physiologically regulated by external stimuli such as lemon juice suggesting the final release of NO to be under neural control.

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MATERIALS AND METHODS

Saliva donors: male or female healthy adults with no medical problems were selected for these studies.

Collection of saliva: for basal measurements saliva was collected after the donor rinsed the mouth with an antiseptic (Peridex) in order to reduce bacterial contamination. The samples were collected within the first 30-60 seconds, usually 200-300 µl were collected and refrigerated until assayed. For stimulated NO production donors after basal collection were given 1 ml of lemon juice to rinse their mouths, and then rinse their mouths out with water following which saliva was collected immediately and five minutes later and handled as described.

Nitrite analysis: NO_2 measurement by the Greiss reagent (consisting of equal volumes of 0.1% N-(1-naphthylyl)-ethylenediamine HCL and 1% sulfanilamide plus 5% H_3PO_4) was taken as a reflection of NO generation as described previously (3). Briefly, 50 µl aliquotes of the saliva sample in triplicates from each donor was incubated with an equal volume of the Greiss reagent in a 96 well U bottom microtiter plate for 5 min at room temperature. With each study a standard curve was established using $NaNO_2$ in a range between 10 and 300 mM for each assay. After a 10 min. reaction at room temperature the microplate was read on a spectrophotometer at 545 nm and the data derived from the slope of the $NaNO_2$ curve presented as the median of triplicates.

RESULTS AND DISCUSSION

Preliminary experiments revealed the presence of NO_2 in human saliva. Initially we were concerned about the source of the NO, especially the possible role of oral bacteria in the production or induction of NO. In order to overcome this problem we used an oral antiseptic (Peridex) in order to minimize this possible source. Our studies revealed the presence of NO as measured through NO_2 in freshly secreted saliva in all individuals tested (Table 1) albeit in variable quantities. Furthermore, we studied the effect of stimulation of salivary flow on NO production by using diluted lemon juice as the stimulant. The results of such experiments (Fig. 1) from six individuals revealed a fall from basal levels in five of the six donors soon after stimulation ie within the first sixty seconds, but quickly rose at least to basal or suprabasal levels in five minutes following stimulation.

The cellular origin, the physiological and possible pathological role of NO in saliva is at present unknown. NO is produced from L-arginine in a number of tissues of disparate derivation such as neurons, endothelial cells, and macrophages. Salivary secretion contains a large array of electrolytes, glycoproteins, enzymes and growth factors. The secretory responses of the salivary glands are mediated by multiple stimuli such as sight, smell, chemical and physical factors in the meal (4). The CNS is the final pathway of execution of all these effects thorough both parasympathetic/cholinergic and also non-cholinergic / sympathetic nerve endings (5). Immunostaining of these nerve endings has revealed the presence of VIP and substance P in the nerve fibers of the salivary glands (4). The stimulation data from our experiments suggest that NO secretion in the salivary glands might be regulated by neural mechanisms along with control of salivary flow (5, 6).

The cellular source of salivary NO is at present unknown. There are at least four possible sources, namely: nerve endings, salivary gland secretory cells, salivary gland

TABLE 1. Basal salivary NO_2 levels in twenty five healthy individuals

Donor number	NO_2 levels (mM)*
1.	16.18
2.	18.66
3.	18.88
4.	22.45
5.	23.32
6.	25.36
7.	28.86
8.	28.88
9.	39.94
10.	44.90
11.	52.48
12.	64.72
13.	65.89
14.	72.21
15.	74.93
16.	84.50
17.	85.71
18.	127.51
19.	139.54
20.	168.48
21.	250.73
22.	237.87
23.	284.91
24.	307.99
25.	396.95

* See materials and methods for assay.

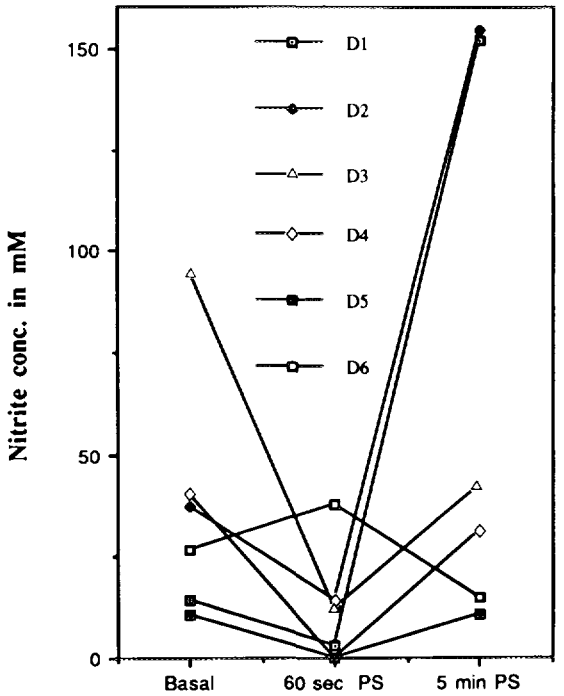

FIGURE 1. Effect of lemon juice stimulation on salivary NO_2 production. Basal saliva samples were collected and then donors were given lemon juice and saliva samples collected immediately, i.e., within 60 seconds post stimulation (60 sec PS) and again at 5 minutes post stimulation (5 min PS) and assayed for NO_2 levels as described in materials and methods.

endothelial cells, or macrophages in response to oral bacterial products. However, the data from our experiments indicationg a role for neural control suggest the saliuvary glandular cells as the possible source of the NO. Finally, the role of NO in saliva is unclear. NO being a highly reactive radical may participate in the non-specific natural defense mechanisms of the oral cavity to prevent bacteria from overgrowing or alternatively NO may also play a role in the maintenance of mucosal integrity and homeostasis and protection from potential carcinogens with which it can form nontoxic complexes.

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