Prostaglandin E_1 induced salivary secretion[1]

J.H. Yu, S. Burns and Charlotte A. Schneyer

Department of Physiology and Biophysics, University of Alabama in Birmingham, Birmingham (Alabama 35294, USA), 7 January 1982

Summary. PGE_1 but not $PGF_{2\alpha}$ at 500–1000 µg/kg induced a slow and sparse flow from the parotid and no flow at all from submaxillary glands. Composition of PGE_1-induced parotid saliva was quite different from that evoked by any autonomic agonists. The present study suggests that PGE_1 might act directly on parotid acinar cells.

Secretion of saliva from the salivary glands is generally elicited only in response to stimulation of the autonomic innervations to the glands or to drugs that mimic the action of the autonomic innervations. In addition, it has recently been shown that some peptides, such as substance P[2-4] and lipid-like prostaglandins, on injection, can also cause secretion of saliva[5,6]. For example, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been shown to induce salivation from dog submaxillary gland[5]. However, the composition of saliva evoked by prostaglandins has never been characterized. The present study was undertaken to investigate the effects of various types of prostaglandins such as E and F series on salivary secretion since they have been shown to play important roles in autonomic neurotransmission in a variety of tissues, including salivary glands[7].

Materials and methods. Male Long-Evans rats (350–400 g), 4–6 months old, were used in these experiments. The rats were maintained on lab chow and water ad libitum until 18 h before the experiment, when food but not water was removed. The rats were anesthetized by i.p. injection of sodium pentobarbital (50 mg/kg b.wt). Tracheotomy was performed to provide a clear airway. For collection of submaxillary saliva, a fine polyethylene cannula (Clay-Adams PE 10) was inserted to a distance of about 4 mm in the oral opening of one submaxillary duct. The saliva was collected from the end of the cannula using disposable calibrated micropipettes. Parotid saliva was collected by micropipette directly from the cut end of the gland duct. For intraarterial (i.a.) injection of prostaglandins, the common carotid artery was carefully exposed and separated from the sympathetic nerve trunk and vagus nerve. Slow infusion of the carotid artery was accomplished either by inserting a 30-gauge (o.d. 0.305 mm) needle by a micromanipulator or by inserting a fine polyethylene cannula (Clay-Adams PE 50) into the artery. [Na] and [K] of evoked saliva were determined by flame photometry (Instrumentation Labs, Model 143) with lithium internal standard. Calcium concentrations were measured in an ultramicro-calcium analyzer (Precision Systems). Amylase activity of saliva and glands was determined by the method of Myers et al.[8]. Prostaglandins were purchased from Upjohn Co. Stock solutions of prostaglandins were made in 95% ethanol and kept in the refrigerator. Appropriate dilutions were made fresh in saline as needed.

Results. Since it has been shown that administered prostaglandins are inactivated rapidly by an enzyme present in blood[9], i.a. injection to salivary glands was performed to study the direct effect of prostaglandins on these organs. Slow infusion of prostaglandin E_1 (PGE_1) (10–250 µg/kg) or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (10–500 µg/kg) into the common carotid artery of rats did not elicit any visible salivary flow from either parotid or submaxillary gland. However, i.a. injection of PGE_1 at 500–1000 µg/kg caused a slow and significant salivary flow from the submaxillary gland. Secretion evoked by PGE_1 was authentic and not mechanical since slow infusion of a similar volume of ethanol or varying doses of $PGF_{2\alpha}$ into the same artery did not elicit salivary flow. Composition of PGE_1-evoked parotid saliva was analyzed. For comparison, other known secretagogues were also used. The [Na], [K], [Ca] and amylase activity of such salivas were determined and shown in the table. Composition of saliva evoked by PGE_1 was different from the evoked by adrenergic agonists; [K] and [Ca] of PGE_1-evoked saliva were lower than that evoked by norepinephrine or by the α-adrenergic agonist, phenylephrine or the β-adrenergic agonist, isoproterenol. Amylase activity of PGE_1-evoked saliva, however, resembled that found with phenylephrine stimulation. [Na] and [K] of saliva evoked by phenylephrine, isoproterenol and norepinephrine were similar. Salivary amylase activity was highest with isoproterenol infusion. Norepinephrine elicited the fastest onset of salivary flow but flow lasted less than 10 min. PGE_1, on the other hand, induced a slow onset of salivary flow; however, its action was also short. Salivary flow induced by phenylephrine or isoproterenol also appeared immediately after injection and persisted longer than that observed with norepinephrine or PGE_1. In addition, PGE_1 caused a $9.8 \pm 1.9\%$ (4 rats) depletion of glandular amylase of parotid gland. However, PGE_1 did not alter total glandular Ca concentration of this gland. Thus, before PGE_1 Ca concentration was 11.0 ± 0.5 (3 rats); after PGE_1, 12.6 ± 0.8 (3 rats).

Discussion. Unlike substance P, PGE_1 is not a potent secretagogue. A relatively large dose of PGE_1 is required to evoke a significant salivary flow. While substance P evokes a rapid an copious flow from both parotid and submaxillary glands (unpublished observations), PGE_1 induced a slow and sparse flow from the parotid and no flow at all from submaxillary gland. On the basis of Na, K, Ca and amylase concentration of PGE_1-evoked parotid saliva, it

Composition of parotid saliva evoked by PGE_1 and adrenergic agonists

Secretagogue	Flow rate (µl/min/g wet wt)	Concentration (mEq/l) Na	K	Ca	Amylase activity[a] (mg/µg/saliva)
E_1 (500 µg/kg)	4.4	61–95	25–30	2.58 ± 0.34 (3)	132 ± 33 (7)
Norepinephrine (50 µg/kg)	27.7	97.1 ± 2.3^b (3)	49.6 ± 2.5^c (3)	11.31 ± 0.90^d (3)	292 ± 20^c (3)
Phenylephrine (50–100 µg/kg)	2.9	93.9 ± 5.5 (3)	47.8 ± 1.6^c (3)	7.28 ± 0.11^c (3)	153 ± 13 (3)
Isoproterenol (500 µg/kg)	6.3	113.3 ± 6.8 (3)	46.3 ± 4.3^c (3)	13.90 ± 0.30^d (3)	1026 ± 12^d (3)

All secretagogues were infused intraarterially via the external common carotid artery. Ethanol-alone infusion did not evoke any salivary flow. [a] Amylase activity is expressed as mg reducing substance (as glucose) per 15 min/µl fresh saliva. [b] Values are means ± SE. Numbers in parentheses refer to number of rats. [c] Values are significantly different from those using E_1 ($p < 0.05$); [d] $p < 0.001$.

appears unlikely that PGE_1-induced salivary secretion is the result of neurotransmitter release from postganglionic autonomic nerve endings of parotid gland. However, PGE_1 might act directly on acinar cells of this gland. Although PGE_1 at lower doses did not evoke salivary secretion, it had a modulating effect on secretory responses evoked by parasympathetic nerve stimulation of the parotid[7].

1 Acknowledgment. This work was supported by NIDR grant DE05633. The authors wish to thank Ms Sonya Wynn for her technical assistance.

2 S.E. Leeman and R. Hammerschlag, Endocrinology *81,* 803 (1967).
3 V. Erspamer, L. Negri, G. Falconieri-Erspamer and R. Endean, Archs Pharmac. *289,* 41 (1975).
4 V. Erspamer, G. Falconieri-Erspamer and G. Linari, in: Substance P, p. 67. Eds U.S. von Euler and B. Pernow. Raven Press, New York 1977.
5 R.A. Hahn and P.N. Patil, Eur. J. Pharmac. *25,* 279 (1974).
6 N. Taira and S. Satoh, Life Sci. *13,* 501 (1973).
7 C.A. Schneyer and J.H. Yu, Ala. J. med. Sci. *19,* 248 (1982).
8 V.C. Myers, A.H. Free and E.E. Rosinski, J. biol. Chem. *154,* 39 (1944).
9 M. Hamberg and B. Samuelsson, J. biol. Chem. *246,* 6713 (1971).

Synthetic corticoliberin needs arginine vasopressin for full corticotropin releasing activity[1]

J.-L. Bény and A.J. Baertschi

Department of Animal Biology, University of Geneva, CH-1211 Geneva 4 (Switzerland), 30 March 1982

Summary. The corticotropin-releasing activity of synthetic corticoliberin increases 75–90% when it is incubated in the presence of arginine vasopressin with rat anterior pituitary cell suspensions; this suggests a synergistic control of ACTH secretion by vasopressin and corticoliberin.

A synthetic sheep corticoliberin (sCRF) has recently been shown to evoke a marked release of corticotropin (ACTH) in vitro[2] an in vivo[3]. Because of our interest in the role of arginine vasopressin (AVP) in the control of ACTH secretion[4–7], we tested the hypothesis that sCRF and AVP may interact at the anterior pituitary level. Vitamin C has previously been implicated in CRF activity[6,8]; therefore we also asked the question whether vitamin C played a role in the biological activity of sCRF.

Methods and materials. CRF activity of various compounds was determined by the Sayers assay[9], as described previously[5,6], by measuring the amount of bioactive ACTH released during 1 h from dispersed anterior pituitary cells. The 1-ml medium was a Krebs-Ringer bicarbonate buffer supplemented with 2 mg/ml glucose and 1 mg/ml beef serum albumin. Medium from anterior pituitary cell suspensions was tested in duplicate on adrenocortical cells.

Secretagogues included sCRF (Bachem); AVP (Sigma); and basal hypothalamic extracts[6]. The sCRF was diluted under nitrogen in a degassed 0.1 N HCl solution, and one half of the solution was supplemented with 10^{-3} M vitamin C. The 2 types of solutions were distributed in 1 ml aliquots containing 2 µM sCRF, frozen at −20 °C, and neutralized before the assay.

Average CRF activities from 4 separate assays were determined for sCRF, AVP, hypothalamic extracts, vitamin C and combinations of the above at doses similar to those used previously[2,6]. Differences in means were analyzed for statistical significance by Student's t-test for small numbers.

Results and discussion. In contradistinction to the exponential dose-response curves for hypothalamic extracts[5,8,9], those for sCRF display a progressively lower slope at

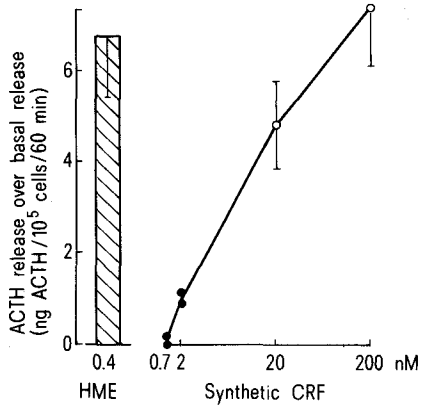

Figure 1. CRF activity of synthetic CRF (sCRF) and of 0.4 equivalents of medial basal hypothalamic extracts (HME). CRF activity is expressed by the amount of ACTH released per 100,000 cells during the 1-h incubation. Basal release, 0.8 ± 0.18 ng/h/10^5 cells, was subtracted to yield the net CRF effect. Averages and SEM are from 4 experiments (2 experiments for the lowest doses of sCRF). The sCRF was conserved in vitamin C.

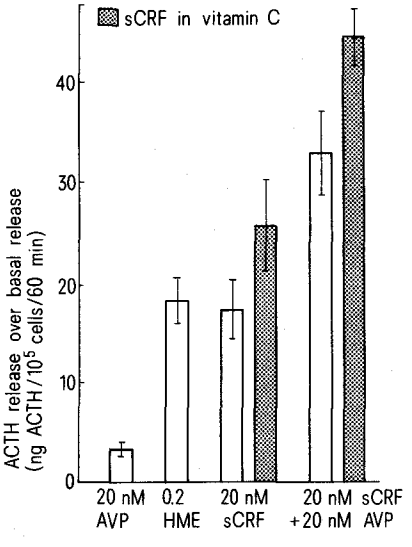

Figure 2. CRF activity of AVP, HME, sCRF and sCRF plus AVP. Means ± SEM are from 4 experiments each in a different (more sensitive) assay series than that in figure 1. CRF activity (see legend to fig. 1). Basal ACTH release was 0.72 ± 0.03 ng/h/10^5 cells. The sCRF was conserved (hatched column) or not (white column) in vitamin C.