Smokeless tobacco potentiates VIP-induced DNA synthesis and inactivates NEP 24.11 in oral keratinocytes

ISRAEL RUBINSTEIN
Department of Medicine, University of Illinois at Chicago; and West Side Department of Veterans Affairs Medical Center, Chicago, Illinois 60612

Rubinstein, Israel. Smokeless tobacco potentiates VIP-induced DNA synthesis and inactivates NEP 24.11 in oral keratinocytes. Am. J. Physiol. Cell Physiol. 278: C391–C396, 2000.—The purpose of this study was to determine whether exposure of cultured chemically transformed hamster oral keratinocytes (HCPC-1) to an aqueous extract of smokeless tobacco (STE) potentiates DNA synthesis elicited by vasoactive intestinal peptide (VIP), an autocrine neuropeptide, and, if so, whether this response is associated with inactivation of neutral endopeptidase 24.11 (NEP 24.11), an ectoenzyme that cleaves and inactivates VIP very effectively, in these cells. I found that STE and VIP each elicited a modest, albeit significant, increase in DNA synthesis in cultured HCPC-1 cells (P < 0.05). However, incubation of HCPC-1 cells with STE together with VIP evoked a significant, concentration-dependent increase in DNA synthesis that was mediated by VIP receptors. The effects of STE and VIP were synergistic. Maximal response was observed after a 48-h incubation. STE significantly attenuated NEP 24.11 activity in HCPC-1 cells at a time when VIP-induced DNA synthesis was maximal. Collectively, these data indicate that STE potentiates VIP-induced DNA synthesis in cultured oral keratinocytes, and that this response is temporally related to STE-induced inactivation of NEP 24.11 in these cells. I suggest that NEP 24.11 modulates the mitogenic effects of smokeless tobacco in the oral epithelium, in part, by inactivating VIP.

Oral mucosa; epithelium; leukoplakia; oral cancer; snuff; 5-bromo-2′-deoxyuridine; peptidase; hamster

IT IS ESTIMATED THAT EVERY YEAR more than 300,000 adolescents become regular users of smokeless tobacco in the United States (8, 47). A growing scientific evidence suggests that regular use of smokeless tobacco is associated with oral mucosa injury and inflammation (12, 14, 18). Importantly, smokeless tobacco may predispose susceptible individuals to oral epithelial cell dysplasia and cancer (14, 18, 28). However, Müns et al. (27) showed that an aqueous extract of smokeless tobacco (STE) has no significant effects on proliferation of cultured chemically transformed golden Syrian hamster oral keratinocytes (HCPC-1) (51). These disparate results suggest that host factors elaborated in the oral mucosa could modulate the deleterious effects of smokeless tobacco.

To this end, the oral epithelium, which is exposed directly to smokeless tobacco, is densely innervated by nerve fibers containing neuropeptides, most notably vasoactive intestinal peptide (VIP), an autocrine neuropeptide (10, 17, 36, 49). Nicotine, a major constituent of smokeless tobacco (18, 33, 34), has been shown to stimulate VIP release from nerves in the rat gastric fundus (5, 25). It is well established that VIP promotes growth of cultured skin keratinocytes and enterocytes (15, 31, 32, 41, 44, 50). However, under normal physiological conditions the biological effects of VIP are short-lived, due, most likely, to proteolytic inactivation and spontaneous hydrolysis of the peptide (26, 30, 35). Hence, for VIP to play a significant role as a growth factor in the oral epithelium, local peptide catabolism has to be slowed.

Previous work from my laboratory showed that neutral endopeptidase 24.11 (NEP 24.11; EC 3.4.24.11), an ectoenzyme widely distributed in the oral epithelium that cleaves and inactivates VIP very effectively in various organs (6, 19, 22, 35, 48, 52), modulates the vasorelaxant effects of VIP in the in situ hamster oral mucosa (43). In addition, Gao et al. (12) showed that short-term suffusion of an aqueous STE on the in situ hamster cheek pouch decreases NEP 24.11 activity in tissue homogenates. Importantly, expression of NEP 24.11 is reduced in stomach, colon, and lung cancer relative to normal tissues and regulates the growth of certain malignant tumors (2–4, 10, 38, 40). For instance, Bunn et al. (2) showed that exogenous NEP 24.11 inactivates autocrine peptides involved in lung cancer signal transduction and inhibits the growth of lung cancer and normal lung cell lines in vitro and in vivo in athymic nude mice.

Taken together, these data suggest that smokeless tobacco placed on the oral mucosa could stimulate nerves to release VIP and inactivate NEP 24.11 in the epithelium. This, in turn, will slow local VIP catabolism thereby promoting epithelial cell growth (36, 37, 42). The purpose of this study was to begin to address this issue by determining whether exposure of HCPC-1 to STE potentiates VIP-induced DNA synthesis and, if so, whether this response is associated with inactivation of NEP 24.11.

METHODS

General

Preparation of STE. The extract was prepared in my laboratory according to the method of Oh et al. (29) as
previously described (11, 12, 27, 37). Briefly, 10 g of smokeless tobacco (153 moist snuff; Tobacco and Health Research Institute, University of Kentucky, Lexington, KY) were mixed with 100 ml DMEM and incubated at 37°C for 2 h. The mixture was then centrifuged at 450 g for 10 min. The supernatant was collected and centrifuged at 13,000 g for 1 h. After adjusting the pH to 7.4 using 0.1 N HCl, the resulting supernatant, designated arbitrarily as 1:1 dilution of raw smokeless tobacco (11, 12, 27, 37), was filtered through a Millipore filter (pore size, 0.45 μm), divided into 2 ml samples, snap-frozen in liquid nitrogen, and stored at −70°C until used.

Culture of oral keratinocytes. 7.12-Dimethylbenz[a]anthracene-transformed golden Syrian HCPC-1 were kindly provided by Dr. D. T. Wong (51). They have been previously used in my laboratory (37). Cells were seeded in microwell, flat-bottom 96-well cell culture cluster (Costar, Cambridge, MA) at a density 30,000 cells/well in 100 μl DMEM supplemented with 10% FCS (GIBCO, Grand Island, NY) and antibiotics (50 μg/ml streptomycin and 2 μg/ml fungizone/ml; GIBCO). The cells were maintained in 95% air-5% CO2 at 37°C for 24–72 h as outlined below. Cell viability was always >95% as determined by morphological examination using phase-contrast microscopy and 0.1% trypan blue dye exclusion test.

Experimental Protocols

Effects of STE and VIP on DNA synthesis in HCPC-1 cells. HCPC-1 cells (30,000 cells/ml) were incubated in the absence and presence of increasing concentrations of STE (1:1,000, 1:500, and 1:100 aqueous dilutions) at 37°C for 72 h. In a second series of experiments, cells were incubated in the absence of STE (1:1,000, 1:500, and 1:100 aqueous dilutions) together with VIP (10^{-6} M) or VIP (10^{-5} M) and STE (1:100 aqueous dilution) for 24–72 h. In another series of experiments, cells were incubated with VIP_{10-28} (10^{-5} M) and STE (1:100 aqueous dilution) for 24–72 h. Incubation of cells with VIP_{10-28} alone at concentrations higher than 10^{-5} M was associated with a significant increase in DNA synthesis (data not shown). In some experiments, HCPC-1 cells were incubated with VIP_{1-12} (10^{-5} M), an inactive peptide fragment, alone and in the presence of VIP (10^{-5} M) and STE (1:100 aqueous dilution) for 24–72 h. Each experiment was conducted in triplicate. The concentrations of STE, VIP, VIP_{10-28}, and VIP_{1-12} used in these experiments are based on preliminary and previous studies in my laboratory and reports in the literature (11, 12, 27, 37, 39, 43).

5-Bromo-2′-deoxyuridine (BrdU) incorporation into the DNA of HCPC-1 cells was used as a proliferative index as previously described for oral keratinocytes by Garlick et al. (13) and Tomakidi et al. (46). A commercially available BrdU-labeled DNA ELISA kit was used according to the manufacturer’s instructions (5-bromo-2′-deoxyuridine-Labeling and Detection Kit III; Boehringer Mannheim, Indianapolis, IN) (20). Absorbency was read at 405 nm using a thermoregulated ELISA microplate reader (SpectraMax 340; Molecular Devices, PaloAlto, CA) and expressed in arbitrary units.

Effects of STE on NEP 24.11 activity in HCPC-1 cells. HCPC-1 cells were seeded in 100-mm tissue culture dishes (Costar) at a density of 1 × 10^5/ml for 24–72 h in the absence and presence of STE (1:100 aqueous dilution). At the end of the incubation period, the cells were scraped from the dish using a rubber policeman and PBS (GIBCO), and cell extracts were prepared as previously described (36). Protein concentration in cell lysates was determined by the method of Bradford (1). NEP 24.11 activity in cell lysates was determined by a sensitive two-stage enzymatic reaction using the synthetic substrate 3-carboxypropanoyl-alanyl-alanyl-leucine-4-nitroanilide supplemented with bacterial (Streptomyces griseus) aminopeptidase I (SGAPI) kindly provided by Dr. S. Blumberg as previously described in my laboratory (21, 48). The assay was performed with 0.4 mM substrate in 50 mM Tris-HCl, 100 mM NaCl, 1 mM CaCl_2, pH 7.5 and 6.7 pg/ml SGAPI in a volume of 200 μl. Incubation was performed in flat-bottom 96-microtiter plates at 23°C for 30 min. NEP 24.11 activity was measured by following the increase in absorbency at 405 nm that is due to the release of p-nitroaniline from the substrate using an ELISA microplate reader. The amount of SGAPI used is optimal for the assay conditions, and its activity is not significantly augmented by the comparatively small amount of endogenous aminopeptidase I present in tissue extracts. Tissue NEP 24.11 activity was expressed as picomoles per minute per milligram protein.

Drugs and chemicals. DMEM was obtained from GIBCO. Human VIP was obtained from American Peptide (Sunnyvale, CA). VIP_{10-28} and VIP_{1-12} were obtained from Sigma Chemical (St. Louis, MO). STE and drugs were diluted in DMEM to the desired concentrations on the day of the experiment.

Data and statistical analyses. Data are expressed as means ± SE. Statistical analysis was performed by two-way ANOVA and the Newman-Keuls test. P < 0.05 was considered significant.

RESULTS

Effects of STE and VIP on DNA Synthesis in HCPC-1 Cells

Incubation of HCPC-1 cells with increasing concentrations of STE alone elicited a small but significant increase in DNA synthesis in HCPC-1 cells relative to media at the highest concentration used (1:100 aqueous dilution; Fig. 1A; each group, n = 4; P < 0.05). Likewise, incubation of HCPC-1 cells with VIP (10^{-7} M) alone evoked a small but significant increase in DNA synthesis relative to media only at a concentration of 10^{-6} M (Fig. 1B; each group, n = 4; P < 0.05). The increase in DNA synthesis during exposure to STE (1:100 aqueous dilution) and VIP (10^{-6} M) alone was maximal after incubation of HCPC-1 cells for 48 h (Fig. 2; each group, n = 4; P < 0.05). By contrast, VIP (10^{-6} M) significantly potentiated STE-induced increase in DNA synthesis at all three concentrations of STE used (Fig. 1A; each group, n = 4; P < 0.05). Likewise, STE (1:100 aqueous dilution) significantly potentiated VIP-induced increase in DNA synthesis at all three VIP concentrations used (Fig. 1B; each group, n = 4; P < 0.05). These effects were also maximal after incubation of STE and VIP together with HCPC-1 cells for 48 h (Fig. 2; each group, n = 4; P < 0.05). The effects of VIP (10^{-6} M) together with STE (1:100 aqueous dilution) on DNA synthesis in HCPC-1 cells were synergetic because BrdU incorporation into DNA evoked by STE together with VIP was greater than the sum of each individual response (Figs. 1 and 2). VIP_{10-28} (10^{-5} M), but not VIP_{1-12} (10^{-5} M), significantly attenuated VIP...
Incubation of HCPC-1 cells in media alone for 24–72 h was associated with a significant, time-dependent decrease in NEP 24.11 activity that was maximal after 48 h (Table 1; each group, n = 4; P < 0.05). Incubation of cells with STE (1:100 aqueous dilution) for 24–72 h was associated with a significant decrease in NEP 24.11 activity relative to media that was maximal after 48 h (Table 1; each group, n = 4; P < 0.05). Enzyme activity began to recover after 72-h incubation of HCPC-1 cells with STE (1:100 aqueous dilution; Table 1; each group, n = 4; P < 0.05 in comparison to 48 h).

**DISCUSSION**

There are two new findings from this study. First, we found that exposure of HCPC-1 to aqueous STE, at noncytotoxic concentrations that have been previously shown to evoke inflammation in the in situ hamster cheek pouch (11, 12), together with VIP, a ubiquitous autocrine neuropeptide in the oral epithelium (17), is associated with significant, concentration-dependent, synergistic increase in DNA synthesis as assessed by BrdU incorporation. These effects were mediated by VIP receptors because VIP<sub>10–28</sub>, a VIP receptor antagonist in the in situ hamster cheek pouch (43), but not (10<sup>-6</sup> M)-induced DNA synthesis (Fig. 3; each group, n = 4; P < 0.05). VIP<sub>10–28</sub> (10<sup>-5</sup> M) had no significant effects on STE (1:100 aqueous dilution)-induced response (Fig. 3; P > 0.5). VIP<sub>10–28</sub> (10<sup>-5</sup> M), but not VIP<sub>1–12</sub> (10<sup>-5</sup> M), significantly attenuated VIP (10<sup>-6</sup> M) potentiation of STE (1:100 aqueous dilution)-induced DNA synthesis in HCPC-1 cells (Fig. 3; each group, n = 4; P < 0.05).

**Effects of STE on NEP 24.11 Activity in HCPC-1 Cells**

Incubation of HCPC-1 cells in media alone for 24–72 h was associated with a significant, time-dependent decrease in NEP 24.11 activity that was maximal after 48 h (Table 1; each group, n = 4; P < 0.05). Incubation of cells with STE (1:100 aqueous dilution) for 24–72 h was associated with a significant decrease in NEP 24.11 activity relative to media that was maximal after 48 h (Table 1; each group, n = 4; P < 0.05). Enzyme activity began to recover after 72-h incubation of HCPC-1 cells with STE (1:100 aqueous dilution; Table 1; each group, n = 4; P < 0.05 in comparison to 48 h).

**BrdU incorporation into DNA of HCPC-1 cells incubated with STE (1:100 aqueous dilution of raw smokeless tobacco, solid circles), VIP (10<sup>-6</sup> M, open squares), and STE (1:100 aqueous dilution) together with VIP (10<sup>-6</sup> M, solid squares) for 24–72 h. Open circles, media alone. Values are means ± SE; each group, n = 4. *P < 0.05 in comparison to media alone. †P < 0.05 in comparison to STE and VIP alone.**

**Fig. 2.** BrdU incorporation into DNA of HCPC-1 cells incubated with STE (1:100 aqueous dilution of raw smokeless tobacco, solid circles), VIP (10<sup>-6</sup> M, open squares), and STE (1:100 aqueous dilution) together with VIP (10<sup>-6</sup> M, solid squares) for 24–72 h. Open circles, media alone. Values are means ± SE; each group, n = 4. *P < 0.05 in comparison to media alone. †P < 0.05 in comparison to VIP alone.
Table 1. Effects of smokeless tobacco extract on neutral endopeptidase 24.11 activity in HCPC-1 cells

<table>
<thead>
<tr>
<th>Incubation Time:</th>
<th>Neutral Endopeptidase 24.11 Activity, pmol·min⁻¹·mg protein⁻¹</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>125.8 ± 2.5</td>
</tr>
<tr>
<td>24 h</td>
<td>72.0 ± 2.6⁶</td>
</tr>
<tr>
<td>48 h</td>
<td>31.8 ± 3.4⁹</td>
</tr>
<tr>
<td>72 h</td>
<td>35.5 ± 4.7⁶</td>
</tr>
<tr>
<td>Cells and media</td>
<td>78.3 ± 3.9⁶</td>
</tr>
<tr>
<td>alone</td>
<td>33.3 ± 4.7⁶</td>
</tr>
<tr>
<td>Cells and STE</td>
<td>12.3 ± 1.5⁶</td>
</tr>
<tr>
<td>(1:100)</td>
<td>19.8 ± 3.8⁶</td>
</tr>
</tbody>
</table>

Values are means ± SE of 4 experiments in triplicate. STE (1:100), 1:100 aqueous dilution of smokeless tobacco extract. *P < 0.05 in comparison to time 0. ⁶P < 0.05 in comparison to 24 h incubation of cells in media. ⁷P < 0.05 in comparison to time 0. ⁸P < 0.05 in comparison to 48 h incubation of cells in media. ⁹P < 0.05 in comparison to 24 h incubation of cells and STE and 48 h incubation of cells in media, respectively.

VIP1–12, an inactive peptide fragment (43), significantly attenuated VIP-induced increase in DNA synthesis in the absence or presence of STE. Second, STE inactivated NEP 24.11, an exoenzyme widely distributed in oral epithelium that cleaves and inactivates VIP very effectively (12, 35, 43, 52), in HCPC-1 cells after 48-h incubation, a time when VIP-induced DNA synthesis was maximal. This process appears to be reversible after 72 h.

These data suggest that inactivation of NEP 24.11 amplifies VIP-induced DNA synthesis in HCPC-1 cells. This contention is supported, in part, by the studies of Suzuki et al. (43) and Gao et al. (11) who showed that selective inhibitors of NEP 24.11 amplify VIP-induced vasodilation and STE-induced increase in clearance of macromolecules from the in situ hamster cheek pouch, respectively. Moreover, Burns et al. (3) showed recently that pharmacologic inhibition of NEP 24.11 in cultured breast cancer cells amplifies bombesin-induced cell growth. Importantly, Ganju et al. (10) showed that cultured airway epithelial cells grow more rapidly when NEP 24.11 is inhibited. Conversely, exogenous NEP 24.11 inhibits growth of lung cancer and normal lung cell lines in vitro and in vivo athymic nude mice (2, 4). On balance, these data suggest that NEP 24.11 modulates the mitogenic effects of smokeless tobacco and VIP in oral keratinocytes.

It is not feasible to expose HCPC-1 cells to raw smokeless tobacco because of cytotoxicity (18, 27–29, 37). Hence, cells are exposed to a diluted aqueous extract of raw smokeless tobacco. This approach has been previously used to determine the effects of smokeless tobacco on oral keratinocytes (27–29, 37). In addition, several toxic and carcinogenic constituents of aqueous STE used in this study are qualitatively similar to those of raw smokeless tobacco (18, 34). People place smokeless tobacco on the oral mucosa where it is continuously mixed with and diluted in saliva, thereby producing, in essence, an aqueous extract in the vicinity of oral keratinocytes. Collectively, these data indicate that the use of an aqueous extract of raw smokeless tobacco in this study is appropriate.

The chemical composition of smokeless tobacco is complex (34). Consequently, it is difficult to identify constituents of STE that could stimulate DNA synthesis and inactivate NEP 24.11 in cultured HCPC-1 cells. Nonetheless, the role of nicotine, a major constituent of smokeless tobacco that has been shown to stimulate VIP release from nerves in the rat gastric fundus (5, 18, 25, 34), should be considered. Ringdahl et al. (33) showed that daily application of nicotine on the mandibular lip of rats for 6 wk is not associated with oral keratinocyte proliferation. Similar observations were reported by Thelig et al. (45) using cultured human skin keratinocytes. Whether nicotine inactivates NEP 24.11 has not been determined in these studies. Clearly, additional studies are warranted to characterize constituents of STE that amplify VIP-induced DNA synthesis and inactivate NEP 24.11 in oral keratinocytes.

The intracellular signal transduction mechanism(s) underlying STE potentiation of VIP-induced DNA synthesis and inactivation of NEP 24.11 in HCPC-1 cells was not elucidated in this study. Conceivably, membrane-bound NEP 24.11 could downregulate VIP-induced calcium flux and activation of protein kinase C, an important intracellular signal transduction pathway involved in cell growth (36, 42), and/or decrease apoptosis of oral keratinocytes (9, 42). For instance, Cohen et al. (4) showed that exogenous NEP 24.11 attenuates intracellular calcium flux elicited by gastrin-releasing peptide and bradykinin, two autocrine peptides, in H345 lung cancer cell line. In addition, pharmacologic inhibition of NEP 24.11 augmented gastrin-releasing peptide– and bradykinin-induced responses in these cells.

Certain constituents of STE may stimulate HCPC-1 cells to elaborate reactive oxygen species that inactivate NEP 24.11 (28, 37). This contention is supported, in part, by the study of Dusser et al. (7) who showed that cigarette smoke inactivates airway NEP 24.11 due, most likely, to the effect of reactive oxygen species. Exposure to STE may also increase the number and/or affinity of VIP receptors on oral keratinocytes thereby potentiating VIP-induced responses (39).

Irrespective of NEP 24.11 downregulation, the cell growth-promoting effects of VIP are tissue and species specific because VIP has been shown to inhibit human small-cell lung cancer and airway smooth muscle proliferation and to have no significant effects on human lung fibroblast proliferation (16, 23, 24). Additional studies using molecular, biochemical, and cell biology techniques are indicated to elucidate the mechanisms mediating the disparate effects of VIP on cell growth.

In summary, we found that STE potentiates VIP-induced DNA synthesis in cultured oral keratinocytes and that this response is temporally related to STE-induced inactivation of NEP 24.11 in these cells. We suggest that NEP 24.11 modulates the mitogenic effects of smokeless tobacco in the oral epithelium, in part, by inactivating VIP.

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Address for reprint requests and other correspondence: I. Rubinstein, Dept. of Medicine (M/C 787), University of Illinois at Chicago, 840 South Wood St., Chicago, IL 60612–7323 (E-mail: IRubinstein
@uic.edu).

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REFERENCES

inhibition, and experimental and clinical pharmacology. Pharma-
36. Rozengurt, E. Neuropeptides as cellular growth-factors—role of
Smokeless tobacco-exposed oral keratinocytes increase macromo-
lecular efflux from the in situ oral mucosa. Am. J. Physiol.
38. Sato, Y., F. Itoh, Y. Hinoda, Y. Ohe, N. Nakagawa, R. Ueda, A.
Yachi, and K. Imai. Expression of CD10/neutral endopeptidase
in normal and malignant tissues of the human stomach and
colon. J. Gastroenterol. 31: 12–17, 1996.
Ikezaki, and I. Rubinstein. Mechanisms of vasodilation elic-
ted by VIP in sterically stabilized liposomes in vivo. Am. J.
Physiol. Regulatory Integrative Comp. Physiol. 273: R287–R292,
1997.
Hersh, H. Stein, M. E. Sunday, and E. L. Reinherz. CD10/
neutral endopeptidase 24.11 hydrolyzes bombesin-like peptides
and regulates the growth of small cell carcinoma of the lung.
41. Simopoulos, C., J. D. Gaffen, and A. Bennett. Effects of
gastrointestinal hormones on the growth of human intestinal
42. Sugerman, P. B., B. K. Joseph, and N. W. Savage. Review
article: the role of oncogenes, tumor suppressor genes and growth
factors in oral squamous cell carcinoma: a case of apoptosis vs.
Neutral endopeptidase modulates VIP-induced vasodilation in
hamster cheek pouch vessels in situ. Am. J. Physiol. Regulatory
Integrative Comp. Physiol. 271: R393–R397, 1996.
44. Takahashi, K., S. Nakanishi, and S. Imamura. Direct effects of
cutaneous neuropeptides on adenylyl cyclase activity and prolif-
eration in a keratinocyte cell line: stimulation of cyclic AMP
formation by CGRP and VIP/PHM, and inhibition by NPY
through G protein-coupled receptors. J. Invest Dermatol. 101:
45. Theilig, C., A. Brend, A. Ramirez-Bosca, F. F. Gómár, J.
Bereiter-Hahn, B. Keller-Stanislawski, A. C. Sewell, N.
Rietbrock, and H. Holzmann. Reactions of human keratin-
cytes in vitro after application of nicotine. Skin Pharmacol. 7:
Histomorphological and biochemical differentiation capacity in
organotypic co-cultures of primary gingival cells. J. Periodontol.
47. Tomar, S. L., and G. A. Giovino. Incidence and predictors of
smokeless tobacco use among US youth. Am. J. Public Health 88:
48. Vishwanatha, J. K., R. G. Davis, S. Blumberg, X.-P. Gao,
and I. Rubinstein. Increased tissue neutral endopeptidase
24.11 activity in spontaneously hypertensive hamsters. Am. J.
49. Widdicombe, J. G. Autonomic regulation. i-NANC/e-NANC.
Wetzker. Vasoactive intestinal peptide and epidermal growth
factor: co-mitogens or inhibitors of keratinocyte proliferation in
Chiang, K. Matossian, G. Gallagher, and G. Sklar. Detection
of Ki-ras messenger RNA in normal and chemically transformed
Rubinstein. Immunoreactive neutral endopeptidase is de-
creased in uvula epithelium of patients with obstructive sleep