Proceedings of the National Science Council, ROC
Part B: Life Sciences
Vol. 21, No. 4, 1997. pp. 129-136

Effect of Betel Quid on Catecholamine Secretion from Adrenal Chromaffin Cells

CHIN-KUN WANG*,+ AND LUCY SUN HWANG**
**Graduate Institute of Nutritional Science*
Chung Shan Medical and Dental College, Taichung, Taiwan, R.O.C.
***Graduate Institute of Food Science and Technology*
National Taiwan University, Taipei, Taiwan, R.O.C.

(Received March 3, 1997; Accepted June 6, 1997)

ABSTRACT

Health damage and environmental pollution are serious problems caused by betel quid chewing in Taiwan. Many people acquire the habit of chewing betel quid due to its physiological effects, including increased stamina and a general feeling of well-being. In this study, a sympathetic model system of adrenal chromaffin cells and sensory evaluation were used to examine the physiological effects of betel quid and the interaction of all the ingredients (areca fruit, *Piper betle* inflorescence and red lime paste) in betel quid. Physiological effects of cardioacceleration, a slightly drunk feeling, sweating and salivation occurred during the chewing of betel quid (a mixture of areca fruit, *Piper betle* inflorescence and red lime paste) and a mixture of areca fruit and red lime paste. Both induced much more basal catecholamine secretion from adrenal chromaffin cells than did other ingredients and combinations of ingredients. It was evident that the responses in the sympathetic model system were closely correlated with the physiological feeling of well-being. The inhibitory effects of all the chewing juices on catecholamine secretion evoked by carbachol and a high concentration of potassium (high K^+) showed that they perhaps affected the calcium influx through voltage-sensitive channels or the steps involved in secretion after calcium entry to stimulate basal catecholamine secretion from chromaffin cells.

Key Words: betel quid; adrenal chromaffin cells; catecholamine secretion.

I. Introduction

Betel quid, the most popular masticatory in Taiwan, is primarily composed of green areca fruit, *Piper betle* (inflorescence or leaf) and slaked lime paste. The slaked lime, which is handled in the form of a paste, is either white lime, containing no additives, or red lime, which has catechu, an extract of *Acacia catechu* (Chen *et al.*, 1984). Red lime betel quid, containing green areca fruit, *Piper betle* inflorescence and red lime paste, is the main such product consumed in Taiwan (about 70% of all betel quid). People place a whole betel quid into their mouth and macerate it by biting for approximately 2-3 min; they then spit out the initial red chewing saliva of betel quid, and this leads to serious environmental pollution. Most people swallow the remaining chewing mixture with their saliva, but the remaining husk residues are finally discarded (Ko *et al.*, 1992). An average of 14-23 betel quids are chewed per day by one Taiwanese chewer, which is high compared to the amount consumed by chewers in India (Stich *et al.*, 1982) or the Philippines (Stich *et al.*, 1984). Betel quid has long been chewed by people as a stimulant and a pick-me-up owing to its physiological effects, including increased stamina, a general feeling of well-being (Nieschulz, 1967), sweating, salivation, stimulation, cardioacceleration, a slightly drunk feeling and warming of the body and mouth cavity (Hwang *et al.*, 1993). Results from a chromaffin cell model system study and sensory evaluation showed that some physiological effects are mainly produced by arecoline, some by the other three alkaloids (Hwang

+ Graduate Institute of Nutritional Science, Chung Shan Medical and Dental College, 110, Section 1, Chien Kuo North Rd., Taichung, Taiwan, R.O.C. Tel: 886-4-3846164; Fax: 886-4-3890964; e-mail: wck@mercury.csmc.edu.tw

et al., 1993), and others by phenolic compounds (Hwang *et al.*, 1992).

Chromaffin cells, the major cell type in adrenal medulla, have been employed to study stimulus-secretion coupling and other functional properties (Douglas, 1968; Brooks, 1977; Schneider *et al.*, 1977; Waymire *et al.*, 1977; Fenwick *et al.*, 1978; Hersey *et al.*, 1979; Livett *et al.*, 1979; Mizobe *et al.*, 1979; Schneider *et al.*, 1979). Since bovine chromaffin cells are easy to isolate in large quantities and are stable in culture medium for about two weeks (Wilson, 1987), they are one of the most favorable model systems used for the study of the mechanism of stimulus-secretion coupling.

In this laboratory, bovine adrenal chromaffin cells have been used as a model system to study the physiological effects of alkaloids and phenolics from betel quid. In this paper, the multiple effects of mixtures of different ingredients of red lime betel quid on catecholamine secretion and the possible mechanisms of these actions are discussed.

II. Materials and Methods

1. Materials

Fresh areca fruit and *Piper betle* inflorescence were purchased from a farm in Nantou County, Taiwan. They were either used immediately or stored at 4°C until use. Red lime paste, a mixture of catechu (an extract prepared from the heartwood of *Acacia catechu*), various herb extracts and slaked lime were purchased in Taichung city, Taiwan. Betel quid was composed of green areca fruit, *Piper betle* inflorescence and red lime paste (80.5: 12.5: 7, weight ratio).

2. Chemicals

Chemicals were obtained from the following sources. Collagenase (type I) was purchased from the Worthington Biochemical Corporation (NJ, USA). Deoxyribonuclease I and carbachol (carbamylcholine) were obtained from the Sigma Chemical Co. (MO, USA). Dulbecco's Modified Eagle's Medium (DMEM) powder was purchased from Gibco BRL Life Technologies Co. (NY, USA). [^{3}H]-Norepinephrine was obtained from New England Nuclear (MA, USA). Glucose and other salts were obtained from Merck (Darmstadt, FRG).

3. Solutions

The compositions of the various buffers used in this study were as follows: The loading buffer consisted of 137 mM sodium chloride, 4.4 mM potassium chloride, 1.2 mM potassium dihydrogen phosphate, 2.2 mM calcium chloride, 0.7 mM magnesium chloride, 10 mM glucose, 3.6 mM sodium hydrogen carbonate, and 5 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), pH 7.4. The perfusion buffer contained 145 mM sodium chloride, 5.4 mM potassium chloride, 1.0 mM sodium dihydrogen phosphate, 11.2 mM glucose, 100 units/ml penicillin G, 40 μg/ml gentamycin, and 15 mM HEPES, pH 7.4. High K^+ contained 98.2 mM sodium chloride, 56 mM potassium chloride, 2 mM calcium chloride, 0.7 mM magnesium chloride, 11.2 mM glucose, and 10 mM HEPES, pH 7.4. The carbachol solution consisted of 0.3 mM carbachol in loading buffer with 0.5% bovine serum albumin (BSA).

4. Collection of Chewing Juices

Chewing juices of betel quid or its ingredients were obtained from ten volunteers after 3 minutes of continuous chewing. The final volume of the chewing juice was adjusted with deionized water to 3 times the initial weight of the areca fruit (or *Piper betle* inflorescence for the mixture of *Piper betle* inflorescence and red lime paste), and the pH and osmolarity were adjusted to 7.4 and 300 ± 5 mOsmol/kg. Serial dilutions of all the chewing juices were obtained by using a loading buffer containing 0.5% BSA.

5. Preparation of the Chewing Juice from Mixtures of Ingredients

Either two or three ingredients of betel quid were combined, the weight ratios were 80.5: 12.5: 7 for areca fruit, *Piper betle* inflorescence and red lime paste, 6.44: 1 for areca fruit and *Piper betle inflorescence*, 11.5: 1 for areca fruit and red lime paste, and 1.79: 1 for *Piper betle* inflorescence and red lime paste. Volunteers chewed various mixtures, and the chewing juice of each mixture was collected and prepared using the procedure described above.

6. Preparation of the Combined Juice

The betel quid ingredients were prepared using the weight ratios described above (for example, 20.125gm of areca fruit, 3.125gm of *Piper betle* inflorescence and 1.75gm of red lime paste). Each prepared ingredient was chewed alone, and the chewing juice was collected. The combined juice was obtained by mixing the chewing juice of each prepared ingredient and treated according to the chewing juice collecting procedure.

7. Isolation of Bovine Chromaffin Cells

Bovine chromaffin cells were isolated using a previously described method with a minor modification (Wilson, 1987). Adrenal glands were first perfused, using a syringe, via the adrenal vein 3 to 5 times with perfusion solution over a period of 30 minutes at room temperature. The glands were then perfused 1 to 3 times with collagenase solution (0.2% collagenase and 0.002% deoxyribonuclease I in perfusion solution). Following perfusion, the medulla was separated from the cortex and cut into small pieces. Minced medulla was then further digested 2 times with collagenase solution at 37°C for 30 minutes each time. The isolated cells were filtered through 250 μ nylon mesh and then collected by centrifugation (2,300 × g) at room temperature. The isolated chromaffin cells were maintained in culture in DMEM supplemented with 5 mM HEPES, 10 μM 5-fluorodeoxyuridine, 10 μM cytosine-β-D-arabinofuranoside, 44 mM sodium hydrogen carbonate, 10^5 units/L penicillin G, 40 mg/L gentamycin, 10% fetal calf serum and 250 μM ascorbic acid (pH 7.4). Cells are cultured on 96-well culture plates at a density of 2×10^5 cells/well and used for measurements of catecholamine secretion within 3-5 days after plating.

8. Catecholamine Secretion

Cultured cells were incubated for 1 hour at 37°C with 0.65 μM [^{3}H]-norepinephrine (1.54 Ci/mmol) in loading buffer containing 1% ascorbic acid and 0.5% BSA (Kilpatrick *et al.*, 1980). Free [^{3}H]-norepine phrine was then removed by washing the cells 3 times, for 15 minutes each time, with loading buffer containing 0.5% BSA. [^{3}H]-norepinephrine loaded chromaffin cells were stimulated by various secretagouges in the presence or absence of chewing juices as described in the text. After 10 minutes, the supernatant was removed, and a solution of 0.1% Triton X-100 and 2 mM ethylene glycol-bis-(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) was added to the pellet. The radioactivity of both the supernatant (S) and pellet (P) was counted and used to calculate the percentage of the total radioactivity of the cells, which were secreted. Unless otherwise indicated, data are mean ± S. D. of at least three experiments using different batches of cells, each performed in triplicate. The percentage of catecholamine secretion (% of total content) was defined as (S/S+P) × 100%. The net percentage of catecholamine secretion stimulated by various chewing juices were expressed as [(S/S+P) × 100% of experiment] –[(S/S+P) × 100% of control]. Catecholamine secretion induced by carbachol or high K^+ was also used as control, and a mixture of various chewing juices and carbachol or high K^+ was used to determine the means by which all the chewing juices induced catecholamine secretion. The effect of chewing juices on catecholamine secretion evoked by carbachol or high K^+ was expressed as {[(S/S+P) × 100% of experiment] – [(S/S+P) × 100% of control]} / [(S/S+P) × 100% of control] × 100%.

9. Sensory Evaluation

Each ingredient and mixtures of either two or three ingredients of betel quid were prepared according to the ratios described above. The physiological characteristics were evaluated by a 10-member panel which evaluated samples for physiological responses on a six-point scale according to the method of Larmond (Larmond, 1982).

10. Statistical Analysis

Comparisons of the catecholamine secretion induced by all the prepared chewing juices were evaluated by analysis of variance (ANOVA), followed by Duncan's new multiple range test to separate the means of the same dilution factor from various chewing juices. Statistics were obtained using the Statistical Analysis System (SAS Institute, Inc., Cary, NC).

II. Results

1. Catecholamine Secretion

To determine the molecular mechanism of betel quid action, the adrenal chromaffin cells were used as a model system. The saliva alone did not stimulate basal catecholamine secretion from chromaffin cells as compared with the loading buffer control group (data not shown). Fig. 1A shows the effects of various dilutions of chewing juices from areca fruit and red lime paste on basal catecholamine secretion by cultured adrenal chromaffin cells. Total basal secretion decreased with an increasing dilution factor of chewing juice. The effect of the mixture chewing juice on basal secretion was significantly greater than that of either combined juice or the chewing juice of areca fruit ($p < 0.01$). For the combination of areca fruit and *Piper betle* inflorescence, there was no difference in basal secretion between the mixture chewing juice and the combined juice. However, their effects were significantly higher than that of the chewing juice of areca fruit (Fig. 2A). For the combination of *Piper betle* inflorescence and red lime paste, the basal

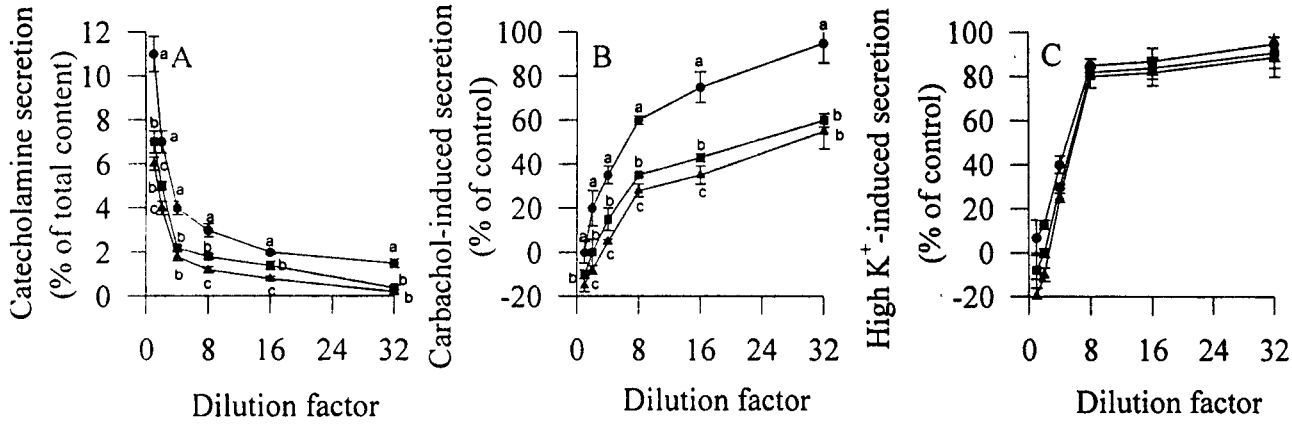

Fig. 1. Effect of mixture of areca fruit and red lime paste on catecholamine secretion, the basal of control was 3.4% (A), on carbachol-induced catecholamine secretion, in the absence of mixture was 7.3% which was used as 100% (B), and on high K^+-induced catecholamine secretion, in the absence of mixture was 4.3% which was used as 100% (C): chewing juice of mixture of areca fruit and red lime paste (circles), combined juice of areca fruit and red lime paste (squares) and chewing juice of areca fruit (triangles). Data bearing different letters in the same dilution factor were significantly different ($p < 0.05$).

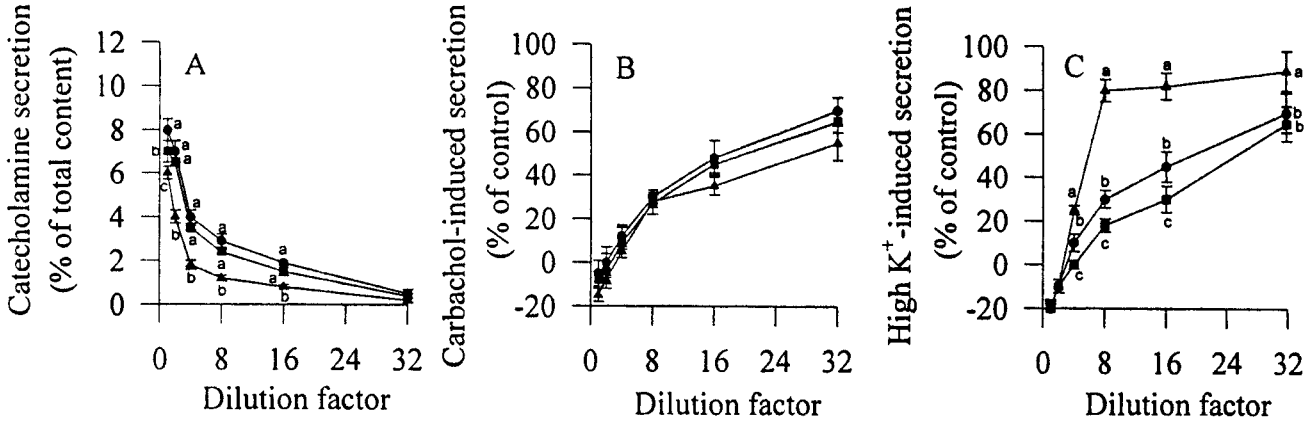

Fig. 2. Effect of mixture of areca fruit and *Piper betle* inflorescence on catecholamine secretion, the basal of control was 3 2% (A), on carbachol-induced catecholamine secretion, in the absence of mixture was 7.6% which was used as 100% (B), and on high K^+-induced catecholamine secretion, in the absence of mixture was 4 3% which was used as 100% (C): chewing juice of mixture of areca fruit and *Piper betle* inflorescence (circles), combined juice of areca fruit and *Piper betle* inflorescence (squares) and chewing juice of areca fruit (triangles) Data bearing different letters in the same dilution factor were significantly different ($p < 0.05$).

secretion induced by the mixture chewing juice was similar to that of the combined juice. However, their effects were significantly higher than that of the chewing juice of *Piper betle* inflorescence ($p < 0.05$; Fig. 3A). For the combination of areca fruit, *Piper betle* inflorescence and red lime paste, the basal catecholamine secretion induced by the mixture chewing juice was much higher than that of the combined juice ($p < 0.01$; Fig. 4A).

In the dilution factor range (1 to 30), Fig. 1B, 2B, 3B, and 4B show that all the different chewing juices inhibited carbachol-induced secretion of catecholamine in a concentration dependent manner. All the chewing juices also depressed high K^+-evoked catecholamine secretion significantly with a concentration dependence similar to that for carbachol-induced catecholamine secretion (Fig. 1C, 2C, 3C, and 4C).

2. Sensory Evaluation

The sensory characteristics of all the prepared components of betel quid are described in Table 1. Mixtures containing *Piper betle* inflorescence gave pungency, warming, fragance and sweating. The physiological effects of cardioacceleration, a drunk feeling, increased stamina, sweating, warming and salivation occurred during the chewing sessions of both whole betel quid (a mixture of areca fruit, *Piper betle* inflo-

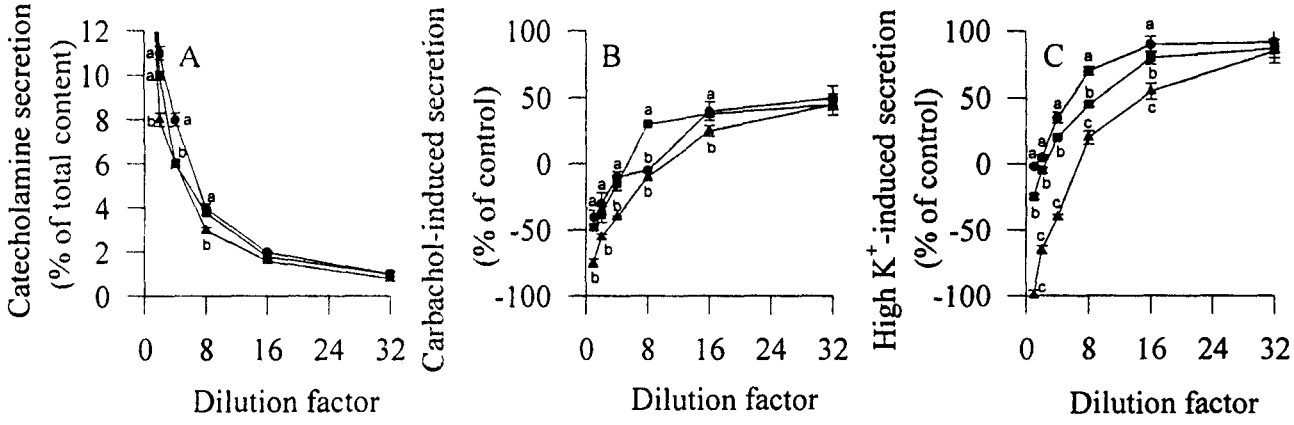

Fig. 3. Effect of mixture of *Piper betle* inflorescence and red lime paste on catecholamine secretion, the basal of control was 4.9% (A), on carbachol-induced catecholamine secretion, in the absence of mixture was 11.6% which was used as 100%, (B) and on high K^+-induced catecholamine secretion, in the absence of mixture was 7.4% which was used as 100% (C): chewing juice of mixture of *Piper betle* inflorescence and red lime paste (circles), combined juice of *Piper betle* inflorescence and red lime paste (squares) and chewing juice of *Piper betle* inflorescence (triangles). Data bearing different letters in the same dilution factor were significantly different ($p < 0.05$).

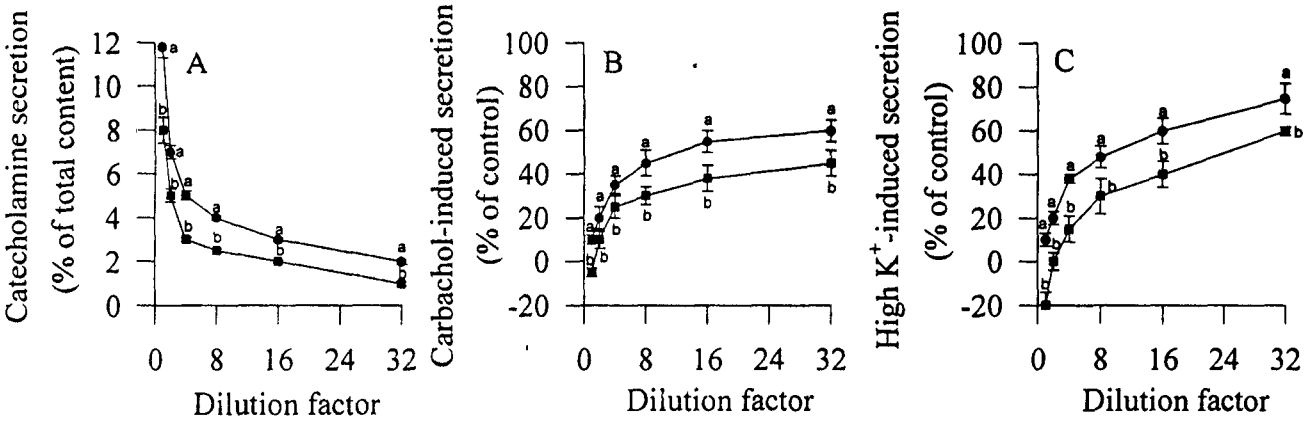

Fig. 4. Effect of mixture of areca fruit, *Piper betle* inflorescence and red lime paste on catecholamine secretion, the basal of control was 9.5% (A), on carbachol-induced catecholamine secretion, in the absence of mixture was 21.0% which was used as 100%, (B) and on high K^+-induced catecholamine secretion, in the absence of mixture was 14.2% which was used as 100% (C): chewing juice of mixture areca fruit, *Piper betle* inflorescence and red lime paste (circles), combined juice of areca fruit, *Piper betle* inflorescence and red lime paste (squares). Data bearing different letters in the same dilution factor were significantly different ($p < 0.05$).

Table 1. Sensory Characteristics of Various Components of Betel Quid

Components*	Sensory Characteristics
AF	astringency, little warming
PI	pungency, warming, fragrance, sweating
RL	fragrance, sweetness
AF+PI	astringency, pungency, warming, sweating
AF+RL	cardioacceleration, slightly drunk feeling, increased stamina, warming, salivation
PI+RL	pungency, warming, fragrance, sweating
AF+PI+RL	cardioacceleration, slightly drunk feeling, increased stamina, warming, fragrance, sweating, salivation

* AF areca fruit
PI *Piper betle* inflorescence
RL Red lime paste
AF+PI mixture of AF and PI at the weight ratio of 6.44: 1
PI+RL mixture of PI and RL at the weight ratio of 1.79: 1
AF+RL mixture of AF and RL at the weight ratio of 11.5: 1
AF+PI +RL mixture of AF, PI and RL at the weight ratio of 80.5: 12.5: 7

rescence and red lime paste) and the mixture of areca fruit and red lime paste.

IV. Discussion

Catecholamine secretion from bovine adrenal medullary chromaffin cells has been studied extensively. The secretory pathway can be dissected into several distinct processes: binding of acetylcholine to its receptor, depolarization of the membrane, an influx of calcium through a voltage-sensitive calcium channel, an increase in cytosolic calcium and exocytosis. The chromaffin cell, therefore, represents a good system for study of the molecular mechanism of drug action (Dogulas, 1975).

The hydrolysis of arecoline has been found to occur during chewing of a mixture of areca fruit and alkaline red lime paste (Nieschulz and Schmersahl, 1968; Wang and Hwang, 1993a). Such hydrolysis was found in chewing juice mixtures containing areca fruit and red lime paste. Arecoline, an agonist for the muscarinic receptor (Taylor, 1990), at 2 mM caused maximum basal catecholamine secretion from chromaffin cells while less secretion occurred when the concentration was higher. Basal secretion of catecholamine evoked by other alkaloids occurred in a dose dependent manner (Hwang *et al.*, 1993). Evidence showed that chewing juice mixtures containing areca fruit and red lime paste induced much more basal secretion. Furthermore, areca fruit, red lime paste and *Piper betle* inflorescence was found to contain some physiologically active phenolic compounds (Wang and Hwang, 1993b; Wang and Lee, 1996) (e.g., eugenol, isoeugenol and hydroxychavicol (Hwang *et al.*, 1992; Wang and Wu, 1996)). The results shown in Fig. 1A-4A indicate that red lime paste and *Piper betle* inflorescence induced high levels of basal secretion. These two ingredients showed strong response in this model system. However, people never chew whole *Piper betle* inflorescence or red lime paste alone owing to their strong responses (e.g., alkalinity, pungency, warming, sweating). The weak responses given by these two ingredients were mainly due to the low levels at which they were added into whole betel quid (20% of the whole betel quid).

Carbachol is a mixed agonist for both nicotinic and muscarinic receptors, and both types of receptors have been shown to affect catecholamine secretion (Kao and Schneider, 1986). The inhibitory effect of all the chewing juices on carbachol-induced catecholamine secretion showed that there could be many routes (for example, by interfering with carbachol binding to receptors, reducing Ca influx, directly affecting the exocytic machinery at the release sites etc.) by which the juices diminished carbachol-induced secretion.

To further understand the mechanism of the basal catecholamine secretion induced by all the chewing juices, the effects of all the chewing juices on high K^+-evoked secretion were also examined. A high concentration of potassium induces catecholamine secretion by depolarizing the chromaffin cells; depolarization causes the voltage-sensitive calcium channel to open and allows calcium influx into the cells. The inhibitory effect of all the chewing juices on high K^+-evoked catecholamine release showed that all the chewing juices induced basal secretion by means of the other steps instead of depolarization.

The inhibition of catecholamine secretion by exocytosis evoked by both carbachol and high K^+ revealed that all the chewing juices may have affected calcium influx through voltage-sensitive channels or the steps of secretion after calcium entry, thus including basal catecholamine secretion from chromaffin cells. Was the basal secretion from the chromaffin cells induced by all the chewing juices of betel quid by way of exocytosis? Further studies on the ^{45}Ca or fura-2 measurements are required to determine whether Ca signals are affected by the chewing juices of betel quid.

The pharmacological properties of betel nut, the dried seed of *Areca catechu*, are usually interpreted in terms of the cholinominetic effects of the major alkaloid component, arecoline. Arecoline has been found to exhibit actions on the central nervous system with the muscarinic property and parasympathetic effects, including tremors and salivation (Leslie, 1965; Nieschuz and Schmersahl, 1968). Similar physiological responses also occurred in this study as determined by sensory evaluation. However, the chromaffin cell study showed that chewing of betel quid activated the sympathetic system, which was different from activation of the parasympathetic system by arecoline. This indicated that betel quid chewing could affect the nervous system in different ways. Further research on the effects of the acting mechanism of betel quid on the nervous system is required.

References

Brooks, J. (1977) The isolated bovine adrenomedullary chromaffin cell; A model of neruonal excitation-secretion. *Endocrinology*, **101:**1369-1378.

Chen, H.C., Chang, Y.S. and Lee, T.C. (1984) The mutagenicity of nitrite-treated aqueous extract of *Piper betle* L. *Proceedings of the National Science Council, ROC: Life Sciences*, **8:**4-10.

Dogulas, W.W. (1968) Stimulus-secretion coupling: the concept and clues from chromaffin cells. *British Journal of Pharmacology*, **34:**451-474.

Dogulas, W.W. (1975) *Handbook of Physiology*, pp.367-388. (Blaschko, H., Sayers, G. and Smith, A.D., Eds.), American Physiological Society, Washington.

Fenwick, E.M., Fajdiga, P.B., Howe, N.B.S. and Livett, B.G. (1978) Functional and morphological characterization of isolated bovine adrenal medullary cells. *Journal of Cell Biology*, **76:**12-30.

Hersey, R.M. and Distefano, V. (1979) Control of phenyl-ethanolamine N-methyltransferase by glucocorticoids in cultured bovine adrenal medullary cells. *Journal of Pharmacology and Experimental Therapeutics*, **209:**147-152.

Hwang, L.S., Wang, C.-K. and Kao, L.-S. (1993) Neuronal activity modulating components in betel quid. Symposium on Betel Quid Chewing and Its Health Effect, Kaoshiung.

Hwang, L.S., Wang, C.-K., Sheu, M.-J. and Kao, L.S. (1992) Phenolic compounds of *Piper betle* flower as flavoring and neuronal activity modulating agents. In: *Phenolic Compounds in Food and Their Effects on Health I*, (Ho, C.T., Lee, C.Y. and Huang, M.T., Eds.), *American Chemical Society Series*, **506:**200-213.

Kao, L.-S. and Schneider, A.S. (1986) Calcium mobilization and catecholamine secretion in adrenal chromaffin cells. A quin-2 fluroescence study. *The Journal of Biological Chemistry*, **261:**4881-4888.

Kilpatrick, D.L., Ledbetter, F.H., Carson, K.A., Kirshiner, A.G., Slepetis, R. and Kirshiner, N. (1980) Stability of bovine adrenal medulla cells in culture. *Journal of Neurochemistry*, **35:**679-692.

Ko, Y.C., Chiang, T.A., Chang, S.J. and Hsieh, S.F. (1992) Prevalence of betel quid chewing habit in Taiwan and related sociodemographic factors. *Journal of Oral Pathology and Medicine*, **21:**261-264.

Larmond, E. (1982) *Laboratory Methods of Sensory Evaluation of Food*, Communications Branch, Agriculture Canada, Otatawa.

Leslie, G.B. (1965) Central stimulant properties of compounds with peripheral muscarinic properties. *Nature*, **208:**1291-1292.

Livett, B.G., Kozousek, V., Mizobe, F. and Dean, D.M. (1979) Substance P inhibits nicotinic activation of chromaffin cells. *Nature*, **278:**256-257.

Mizobe, F., Kozousek, V., Dean, D.M. and Livett, B.G. (1979) Pharmacological characterization of adrenal paraneurons: Substance P and somatostatin as inhibitory modulators of the nicotinic response. *Brain Research*, **178:**555-566.

Nieschulz, O. (1967) Pharmacology of the active principle of betel (I) central effects of arecoline. *Arzneimmttel-Forschung*, **17**:1292-1295.

Nieschulz, O. and Schmersahl, P. (1968) Pharmacology of the active principle of betel (II) conversion of arecoline to arecaidine. *Arzneimmttel-Forschung*, **18:**222-226.

Schneider, A.S., Cline, H.T. and Lemaire, S. (1979) Rapid rise in cyclic GMP accompanies catecholamine secretion in suspensions of isolated adrenal chromaffin cell. *Life Sciences*, **24:**1389-1394.

Schneider, A.S., Herz, R. and Rosenheck, K. (1977) Stimulus-secretion coupling in chromaffin cells isolated from bovine adrenal medulla. *Proceeding of the National Academy of Sciences of USA*, **74:**5036-5040.

Stich, H.F., Rosin, M.P. and Vallejera, M.O. (1984) Reduction with vitamin A and beta-carotene administration of proportion of micronucleated buccal mucosa cells in Asian betel nut and tobacco chewers. *Lnacet*, **1:**1204-1206.

Stich, H.F., Stich, W. and Parida, B.B. (1982) Elevated frequency of micronucleated cells in the buccal mucosa of individuals at high risk for oral cancer: betel quid chewers. *Cancer Letters*, **17:**125-134.

Taylor, P. (1990) Cholinergic agonists. In: *The Pharmacological Basis of Therapeurics*, (Gilman, A.G.,Rall, T.W., Nies, A.S. and Taylor, P, Eds.), Pergamon, New York.

Wang, C.-K. and Hwang, L.S. (1993a) Phenolic compounds of betel quid chewing juice. *Food Science*, **20(5):**514-526.

Wang, C.-K. and Hwang, L.S. (1993b) Analysis of the phenolic compounds in betel quid. *Journal of the Chinese Agricultural Chemical Society*, **31(5):**623-632.

Wang, C.-K. and Lee, W.-H. (1996) Separation, characteristics, and biological activities of phenolics in areca fruit. *Journal of Agricultural and Food Chernistry*, **44(8):**2014-2019.

Wang, C.-K. and Wu, M.-J. (1996) The separation of phenolics from *Piper betle* leaf and the effect on the mutagenicity of arecoline. *Journal of the Chinese Agricultural Chemical Society*, **34(5):**638-647.

Waymire, J.C., Waymire, K.G., Roehme, R., Noritake, D. and Wardell, J. (1977) Regulation of tyrosine hydroxylase by cyclic 3',5' adenosine monophosphate in cultured neuroblastoma and cultured dissociated bovine adrenal chromaffin cell. In: *Structure and Function of Monoamine Enzyme*, pp.327-363. (Usdin, E., Weiner, N. and Youdin, M., Eds.), Marcelol Dekker, New York.

Wilson, S.P. (1987) Purification of adrenal chromaffin cells on Renografin gradients. *Journal of Neuroscience Methods*, **19:**163-171.

檳榔嚼塊對刺激嗜鉻細胞分泌腎上腺素之影響

王進崑 * • 孫璐西 **

* 中山醫學院營養科學研究所
** 臺灣大學食品科技研究所

摘 要

在臺灣，嚼食檳榔已被發現對健康及環境造成很大的影響。許多人喜歡嚼食檳榔是因爲檳榔嚼食後之提神及興奮感覺。本研究利用牛之腎上腺髓質細胞及官能品評，探討嚼食檳榔之生理反應及檳榔嚼塊中各種配料(檳榔果實、荖花及紅灰）間之反應。完整之檳榔嚼塊（檳榔果實、荖花及紅灰）與檳榔果實及紅灰之混合物在咀嚼時有心跳加速、微醉、發汗及增加唾液分泌等反應，而這兩者皆可較單一配料或其他之混合配料誘發嗜鉻細胞分泌更多的腎上腺素。結果顯示，以嗜鉻細胞分泌腎上腺素之交感神經模式系統與人的實際生理反應間有很好的相關性。而檳榔嚼塊對於carbachol與高鉀溶液所誘發之腎上腺素分泌皆具抑制效果，此表示檳榔嚼塊會誘發嗜鉻細胞分泌腎上腺素之作用，可能是發生在細胞發生去極化作用後鈣離子進入細胞之後的步驟上所引起的。