The Journal of Laryngology & Otology (2011), ***125***, *492–496*.

doi:10.1017/S0022215110002574

MAIN ARTICLE

Gamma-aminobutyric acid concentrations in benign parotid tumours and unstimulated parotid saliva

E JEZEWSKA[1,2], A SCINSKA[1,2], W KUKWA[1], A SOBOLEWSKA[3], D TURZYNSKA[3], J SAMOCHOWIEC[4], P BIENKOWSKI[5]

[1]Department of Otolaryngology, Czerniakowski Hospital, Warsaw Medical University, [2]Consultant Otolaryngologist Unit and Departments of [3]Neurochemistry and [5]Pharmacology, Institute of Psychiatry and Neurology, Warsaw, and [4]Department of Psychiatry, Pomeranian Medical Academy, Szczecin, Poland

Abstract

Objective: Apart from its role as an inhibitory neurotransmitter, γ-aminobutyric acid is also thought to regulate various stages of cell proliferation and differentiation in the brain and periphery. The present study aimed to assess the levels of γ-aminobutyric acid and its biochemical precursor glutamic acid (glutamate) in benign parotid tumours and in unstimulated parotid saliva.

Method: Unstimulated parotid saliva was collected bilaterally, using the swab method, in 20 patients with unilateral pleomorphic adenoma or Warthin's tumour. Samples of tumour and adjacent salivary tissue were collected during tumour resection.

Results: Concentrations of γ-aminobutyric acid and glutamate, but not aspartate, were significantly higher in the tumour tissue than in the non-tumour tissue. There was no significant difference in salivary concentrations of γ-aminobutyric acid, glutamate or aspartate, comparing the involved and non-involved side.

Conclusion: The present results provide preliminary evidence that γ-aminobutyric acid may be involved in the growth of benign parotid tumours.

Key words: Parotid Neoplasms; Saliva; Gamma-Aminobutyric acid

Introduction

Gamma-aminobutyric acid (GABA) is a non-proteogenic amino acid present in bacteria, plants and animals.[1] In mammalian cells, GABA is synthesised from glutamic acid (glutamate) by glutamic acid decarboxylase and from polyamines with the aid of diamine oxidase.[1,2] Gamma-aminobutyric acid is a major inhibitory neurotransmitter in the adult mammalian brain, acting at either ionotropic ($GABA_A$ and $GABA_C$) or metabotropic ($GABA_B$) receptors.[1,3,4] Apart from its role as an inhibitory neurotransmitter, GABA is also thought to regulate different stages of brain neurogenesis, including proliferation, migration, differentiation and synaptic integration of newborn neurons.[4,5]

Glutamic acid decarboxylase, diamine oxidase, GABA and GABA receptors can also be present in non-neuronal tissues, including heart, liver, gut, pancreas, salivary glands, ovary and testis.[1,4,6] Although the exact role of GABA in peripheral tissues remains to be established, it is accepted that GABA and GABA receptors are involved in cell proliferation and differentiation.[1,4] Recently, it has been found that GABA negatively regulates proliferation of pluripotent stem cells in peripheral embryonic and adult tissues.[7,8]

In line with the involvement of GABA in neuronal and non-neuronal cell proliferation,[4,5,7,8] it has been reported that GABA levels are significantly higher in malignant tissue of the thyroid,[9] colon[10,11] and mammary glands,[12] compared with normal tissue. Higher GABA levels have also been found in benign colonic adenomas, compared with healthy control tissue.[11] Tatsuta and colleagues[13,14] were the first group to suggest a functional link between GABA and oncogenesis, by showing that GABA and the clinically utilised $GABA_B$ agonist baclofen suppressed gastric carcinogenesis in the rat. More recently, it has been suggested that GABA acts as a near-universal signal which can inhibit the mitotic activity and migration of tumour cells.[6,8] When considering the possible clinical applications of GABA concentration measurement, it is worthy of note that elevated

Accepted for publication 15 July 2010 First published online 17 December 2010

urinary GABA concentrations have been found in ovarian cancer patients.[15]

The parotid gland is the most common site of salivary tumours.[16–18] Eighty per cent of salivary neoplasms occur in the parotid, of which up to 80 per cent are benign. Pleomorphic adenoma is the most common benign parotid neoplasm, comprising approximately 70 per cent of all parotid tumours, and Warthin's tumour is the second most common.[16–18]

To the best of our knowledge, GABA levels have never been assessed in human salivary glands or salivary tumours. Given the above, the aim of the present study was to evaluate GABA concentrations in samples of the most common parotid tumours and adjacent non-tumourous salivary tissue taken during parotid surgery. As GABA can be synthesised from glutamate in the rat parotid gland[19] and in other tissues,[2] we decided to analyse glutamate concentrations in the tumourous and non-tumourous samples. Aspartic acid (aspartate) levels were examined to control for possible non-specific alterations in amino acid concentrations in the tumourous tissue.

Parotid tumours can manifest as abnormalities of the gland itself and/or changes in saliva chemistry.[17] Hence, we decided to assess GABA, glutamate and aspartate levels in unstimulated parotid saliva taken from the same patients before surgery.

The present study was an extension of our previous report showing that the most common benign parotid tumours do not profoundly alter unstimulated saliva secretion from the affected gland.[20]

Materials and methods

Patients

The study was carried out in accordance with the Declaration of Helsinki of the World Medical Association. Each participant signed an informed consent form after study procedures had been fully explained. The protocol for the study was reviewed and approved by the ethics committee of the Warsaw Medical University.

We enrolled in the study patients with primary parotid tumour who were admitted consecutively to the otolaryngology department of the Czerniakowski Hospital for routine surgical procedures. Potential participants were excluded if they had: xerostomia; sialadenitis; sialadenosis; other significant pathology in the head and neck region (except unilateral parotid tumour); or a history of severe liver, renal or endocrine disorder. Patients were also eliminated if they were taking medication with anticholinergic effects or GABA-ergic medication (e.g. benzodiazepines, anticonvulsants and baclofen), as these may affect GABA levels.[15,20,21]

Twenty-five patients were screened for eligibility. Five patients were excluded after initial screening; thus, results from 20 patients are reported. All study participants were Caucasian and aged 18 to 79 years (see Table I for sociodemographic and clinical characteristics). A painless, palpable mass was a major presenting symptom in all cases. There were no signs of facial nerve palsy or local inflammation. The diagnosis of pleomorphic adenoma or Warthin's tumour was confirmed by histopathological examination of the tumour tissue.

Saliva collection

The swab method was used to collect unstimulated parotid saliva from both the involved and non-involved sides.[22] Saliva was collected for 15 minutes with the aid of Salivette cotton rolls (Sarstedt, Rommelsdorf, Germany), as described by Jezewska *et al.*[20] The saliva-soaked rolls were placed in single-use syringes equipped with filter units (0.45 µm; Millex®-HV, Millipore Corporation, Bedfont, Massachusetts, USA) and squeezed until 0.2–0.3 ml of filtered saliva was collected into an Eppendorf tube. Saliva samples were stored at −70°C until further analysis.[23]

Tissue sampling and preparation

Samples of tumour and adjacent salivary tissue were collected from each patient during tumour resection. All specimens were immediately washed in sterile, ice-cold physiological saline (0.9 per cent NaCl), frozen on dry ice and stored at −70°C. Before further analysis, each sample was weighed, placed in an ice-cooled Eppendorf tube and homogenised for 60 seconds in 0.2 M perchloric acid. Homogenates were centrifuged at 10 000 rpm at 4°C for 10 minutes and supernatants filtered through 0.45-µm Millex-HV filters.

Biochemical analysis

The relevant biochemical procedures have been described in detail by Maciejak *et al.*[24] and Scinska-Bienkowska *et al.*[23] Concentrations of GABA, glutamate

TABLE I
BASELINE CHARACTERISTICS OF STUDY PARTICIPANTS

Parameter	Value
Females (%)	40
Age (mean ± SEM (range); years)	48.5 ± 3.9 (18–79)
BMI (mean ± SEM (range))	26.1 ± 1.5 (17.6–40.3)
University degree (%)	35
Presently employed (%)	60
Married or in stable relationship (%)	70
Current smokers (%)	65
Tumour diameter (mean ± SEM (range); cm)	2.2 ± 0.2 (1–4)
Pleomorphic adenomas* (mean ± SEM (range); cm)	2.1 ± 0.1 (1–3)
Warthin's tumours† (mean ± SEM (range); cm)	2.4 ± 0.5 (1.5–4)
Time from tumour onset (mean ± SEM (range); mth)	39.4 ± 11.6 (3–240)

*Total $n = 14$. †Total $n = 6$. SEM = standard error of the mean; BMI = body mass index (kg/m^2); mth = months

https://doi.org/10.1017/S0022215110002574 Published online by Cambridge University Press

and aspartate in saliva and tissue samples were determined with the aid of a gradient reversed-phase high performance liquid chromatography system (Shimadzu, Tokyo, Japan), with pre-column derivatisation with o-phtalaldehyde and electrochemical detection.

Statistical analysis

Differences in GABA, glutamate and aspartate levels were analysed with the aid of Student's *t*-test, unpaired. A p value of less than 0.05 was considered significant.

Results and analysis

Figure 1 shows GABA, glutamate and aspartate concentrations in unstimulated parotid saliva taken from patients with benign parotid tumours. No statistically significant differences were found for GABA ($p = 0.07$), glutamate ($p = 0.4$) or aspartate concentrations ($p = 0.7$), comparing the involved and non-involved sides.

Figure 2 shows tissue concentrations of GABA, glutamate and aspartate. Levels of GABA and glutamate were significantly higher in the tumourous tissue samples compared with the control tissue ($p < 0.01$). There was no significant difference in aspartate concentration between the tumourous and control tissue samples ($p = 0.6$).

Discussion

To the best of our knowledge, this is the first study to show that the most common benign parotid tumours (i.e. pleomorphic adenoma and Warthin's tumour) contain more GABA than healthy parotid tissue. Increased GABA concentrations in the tumour tissue samples were accompanied by a significant rise in glutamate levels but not in aspartate levels. The latter observation suggests that our findings were not

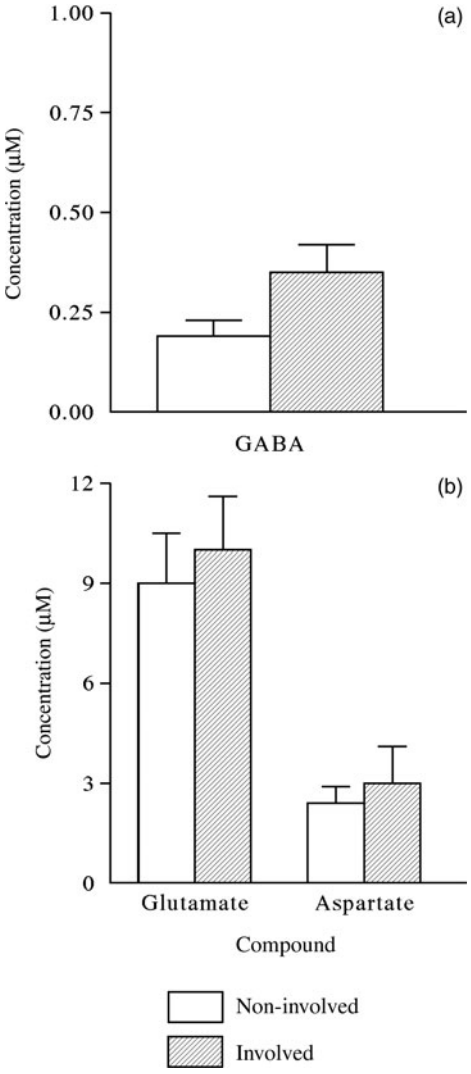

FIG. 1

Concentration of (a) γ-aminobutyric acid (GABA) and (b) glutamate and aspartate, in unstimulated saliva collected from the non-involved (healthy) and involved (tumourous) sides. Bars represent mean values and outliers represent standard error of the mean, for 20 patients with benign parotid tumour.

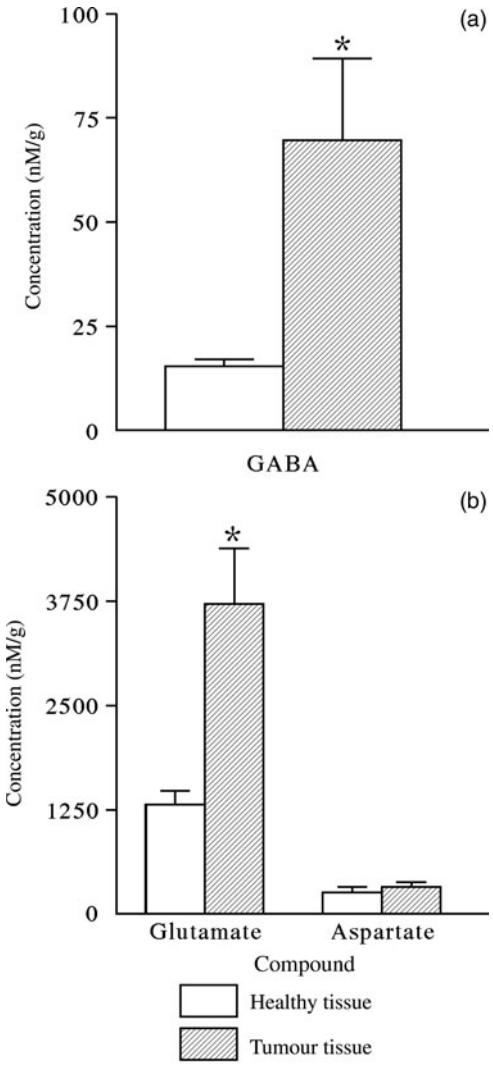

FIG. 2

Concentration of (a) γ-aminobutyric acid and (b) glutamate and aspartate in tumourous and non-tumourous human parotid tissue. Bars represent mean values and outliers represent standard error of the mean, for 20 patients with benign parotid tumour. $^{*}p < 0.01$.

https://doi.org/10.1017/S0022215110002574 Published online by Cambridge University Press

secondary to any non-specific increase in amino acid content of the tumour tissue samples.

There was no statistically significant difference in GABA levels in unstimulated saliva taken from the affected versus unaffected salivary glands. This finding was not completely unexpected, as Warthin's tumours and pleomorphic adenomas are slow-growing tumours with a fibrous capsule of varying thickness,[16–18] and may thus exert little effect on saliva secretion and chemistry. It remains to be established whether salivary GABA levels can rise in patients with malignant parotid tumours.

- **Gamma-aminobutyric acid (GABA) is thought to regulate various stages of cell proliferation and differentiation in the brain and periphery**
- **Gamma-aminobutyric acid may also be a near-universal signal capable of altering tumour cells and modifying mitotic activity**
- **This study assessed GABA, glutamate and aspartate concentrations in benign parotid tumour tissue and unstimulated parotid saliva**
- **Concentrations of GABA and glutamate, but not aspartate, were significantly higher in the tumour *vs* non-tumour tissue samples; no significant difference was found between saliva from the involved *vs* non-involved side**
- **Gamma-aminobutyric acid may be involved in the growth of benign parotid tumours**

Our results provide further support for previous reports[4,6,10–12] (see Introduction) that tumours of various histological origin contain more GABA than healthy tissue. In addition, the present study provides more evidence that GABA levels may rise not only in malignant but also in benign tumours.[11,25] Interestingly, Maemura *et al.*[11] have found a positive correlation between GABA concentration and the degree of atypia in benign colonic tumours. Further studies with larger groups of patients are needed to determine whether GABA concentrations predict the histological characteristics and/or clinical course of parotid tumours.

Sawaki *et al.*[19] have confirmed the presence of GABA and its metabolic enzymes in the rat salivary gland. The present results, taken together with this finding,[19] suggest that the most common parotid tumours contain the molecular machinery required for GABA-ergic transmission. More studies are needed to address this hypothesis and to delineate a possible role for GABA and its receptors in salivary oncogenesis. For example, it may be desirable to assess whether clinically utilised GABA-ergic medications (e.g. baclofen, valproic acid and benzodiazepines) alter the growth of salivary tumours.

Our finding that glutamate concentrations were higher in tumourous than non-tumourous tissue samples was not completely unexpected. Gamma-aminobutyric acid can be synthesised from glutamate by glutamic acid decarboxylase in neuronal and non-neuronal tissues,[1,4] including the rat parotid gland.[19] Thus, increased GABA levels in benign parotid tumours could be secondary to increased substrate (i.e. glutamate) availability. Although glutamate is mainly considered to be a major excitatory neurotransmitter,[26] a large body of evidence has accumulated over the last decade indicating a role for glutamate as an extracellular signal mediator in peripheral autocrine and paracrine systems. Peripheral cells express all the molecular machinery required for glutamatergic transmission, including glutamate receptor and transporter proteins.[26] The present report could be a starting point for further studies on the role of glutamate in signalling cascades regulating salivary oncogenesis.

Acknowledgements

The study was supported by the Warsaw Medical University (EJ, AS and WK) and the Institute of Psychiatry and Neurology, Warsaw, Poland (PB).

References

1 Watanabe M, Maemura K, Kanbara K. GABA and GABA receptors in the central nervous system and other organs. *Int Rev Cytol* 2002;**213**:1–47
2 Tillakaratne NJK, Medina-Kauwe L, Gibson KM. γ-aminobutyric acid (GABA) metabolism in mammalian neural and non-neural tissues. *Comp Biochem Physiol* 1995;**112**:247–63
3 Bormann J. The 'ABC' of GABA receptors. *Trends Pharmacol Sci* 2000;**21**:16–9
4 Watanabe M, Maemura K, Oki K, Shiraishi N, Shibayama Y, Katsu K. γ-aminobutyric acid (GABA) and cell proliferation: focus on cancer cells. *Histol Histopathol* 2006;**21**:1135–41
5 Ge S, Pradhan DA, Ming G-L, Song H. GABA sets the tempo for activity-dependent adult neurogenesis. *Trends Neurosci* 2007;**30**:1–8
6 Ortega A. A new role for GABA: inhibition of tumor cell migration. *Trends Pharmacol Sci* 2003;**24**:151–4
7 Andäng M, Hjerling-Leffler J, Moliner A, Lundgren TK, Castelo-Branco G, Nanou E *et al*. Histone H2AX-dependent $GABA_A$ receptor regulation of stem cell proliferation. *Nature* 2008;**451**:460–4
8 Young SZ, Bordey A. GABA's control of stem and cancer cell proliferation in adult neural and peripheral niches. *Physiology* 2009;**24**:171–85
9 Andersson AC, Henningsson S, Jarhult J. Diamine oxidase activity and γ-aminobutyric acid formation in medullary carcinoma of the thyroid. *Agents Action* 1980;**10**:299–301
10 Kleinrok Z, Matuszek M, Jesipowicz J, Matuszek A, Opolski A, Radzikowski C. GABA content and GAD activity in colon tumors taken from patients with colon cancer or from xenografted human colon cancer cells growing as s.c. tumors in athymic nu/nu mice. *J Physiol Pharmacol* 1998;**49**:303–10
11 Maemura K, Yamauchi H, Hayasaki H, Kanbara K, Tamayama T, Hirata I *et al*. γ-amino-butyric acid immunoreactivity in intramucosal colonic tumors. *J Gastroenterol Hepatol* 2003;**18**:1089–94
12 Mazurkiewicz M, Opolski A, Wietrzyk J, Radzikowski C, Kleinrok Z. GABA level and GAD activity in human and mouse normal and neoplastic mammary gland. *J Exp Clin Cancer Res* 1999;**18**:247–53
13 Tatsuta M, Iishi H, Baba M, Nakaizumi A, Ichii M, Taniguchi H. Inhibition by γ-amino-n-butyric acid and baclofen of gastric carcinogenesis induced by N-methyl-N'-nitro-N-nitrosoguanidine in Wistar rats. *Cancer Res* 1990;**50**:4931–4

https://doi.org/10.1017/S0022215110002574 Published online by Cambridge University Press

14 Tatsuta M, Iishi H, Baba M, Taniguchi H. Attenuation by the GABA receptor agonist baclofen of experimental carcinogenesis in the rat colon by azoxymethane. *Oncology* 1992;**49**: 241–5
15 Nicholson-Guthrie CS, Guthrie GD, Sutton GP, Baenziger JC. Urine GABA levels in ovarian cancer patients: elevated GABA in malignancy. *Cancer Lett* 2001;**162**:27–30
16 Ansari MH. Salivary gland tumors in an Iranian population: a retrospective study of 130 cases. *J Oral Maxillofac Surg* 2007; **65**:2187–94
17 Lee KJ. *Essential Otolaryngology*. Connecticut, USA: Stamford, Appleton & Lange, 1995
18 Madani G, Beale T. Tumors of the salivary glands. *Semin Ultrasound CT MRI* 2006;**27**:452–64
19 Sawaki K, Ouchi K, Sato T, Kawaguchi M. Existence of γ-aminobutyric acid and its biosynthetic and metabolic enzymes in rat salivary gland. *Jpn J Pharmacol* 1995;**67**:359–63
20 Jezewska E, Scinska A, Kukwa W, Bienkowski P. Effects of benign parotid tumors on unstimulated saliva secretion from the parotid gland. *Auris Nasus Larynx* 2009;**36**:586–9
21 Sreebny LM. Saliva in health and disease: an appraisal and update. *Int Dent J* 2000;**50**:140–61
22 Ahlner BH, Lind MG. A swab technique for sialometry. *Acta Otolaryngol* 1983;**95**:173–82
23 Scinska-Bienkowska A, Wrobel E, Turzynska D, Bidzinski A, Jezewska E, Sienkiewicz-Jarosz H *et al*. Glutamate concentration in whole saliva and taste responses to monosodium glutamate in humans. *Nutr Neurosci* 2006;**9**:25–31
24 Maciejak P, Czlonkowska AI, Bidzinski A, Walkowiak J, Szyndler J, Lehner M *et al*. Pregnenolone sulfate potentiates the effects of NMDA on hippocampal alanine and dopamine. *Pharmacol Biochem Behav* 2004;**78**:781–6
25 Okada Y, Taniguchi H, Schimada C. High concentration of GABA and high glutamate decarboxylase activity in rat pancreatic islets and human insulinoma. *Science* 1976;**194**:620–2
26 Hinoi E, Takarada T, Ueshima T, Tsuchihashi Y, Yoneda Y. Glutamate signaling in peripheral tissues. *Eur J Biochem* 2004;**271**:1–13

Address for correspondence:
Dr Przemyslaw Bienkowski,
Department of Pharmacology,
Institute of Psychiatry and Neurology,
9 Sobieskiego St, 02-957 Warsaw, Poland

Fax: +48 22 84 27 644
E-mail: bienkow@ipin.edu.pl

Dr P Bienkowski takes responsibility for the integrity of the content of the paper
Competing interests: None declared