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（出國類別：進　修）

探索大腦－胃腸軸及其相關生化傳遞物質的尋找與定位

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主辦機關:

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關鍵詞: 大腦－胃腸、CRF : cortıcotropın releasing factor、接受器、胃酸、胃運動、低血壓、小腦延髓池、urocortın、人類第二型urocortın。

內容摘要: Cortıcotropın releasing factor (CRF)，是由下視丘側腦室旁的神經核所分泌的41個胺基酸胜　，起初是由於它主要能激活下視丘－腦垂體－腎上腺軸（HPA axıs）對於壓力所產生的反應而有所了解（Nature 1995; 378: 287-292）。CRF胜　家族包含urocortın（Endocrınology 1996, 137: 3896）和urocortın II及urocortın III (Nat Med 2001; 7: 605-611) (Proc Natl Acad Scı 2001; 98: 2843-2848)。CRF及其胜　家族在心臟血管系統、生殖系統、胃腸道、免疫系統和中樞神經系統方面扮演廣泛的角色（Nat Genet 2000; 24: 410-414）(Neuron 1998; 20. 1-20）。因此，CRF家族胜　是科學們探索大腦－胃腸交互作用一個很好的研究物。 CRF和其相關的胜　，其生物作用是經由鍵結至他們的接受器而起動，進一步增加細胞內的cAMP濃度。至今，兩種CRF接受器亞型，CRF1和CRF2，已經在哺乳動物中成功地分離克隆(clone)出來了（Mol Endocrınol 1995; 9: 637-645）。在我給台灣國家衛生研究院2000年的年度結案報告中已經指出，小腦延髓池注射urocortın可經由CRF接受器抑制迷走神經所刺激的胃酸分泌和胃收縮。因此，利用我第一年所得到的經驗，我進一步探索CRF接受器亞型對中樞調節胃酸分泌和胃運動功能的影響。甚至，使用一個全新CRF2選擇性接受器刺激物：人類第二型urocortin（human urocortın II ）去研究週邊CRF2接受器在調節全身血壓的角色。最後，我專注於胃腸CRF2接受器的克隆（clone）、特質分析和鑑定。下面就是我出國至UCLA/CURE進修兩年的計畫總結，同時這些結果也都寫成論文投到有同儕專家審稿（peer-review）的SCI科學期刊：計畫一：研究CRF2接受器在調節小腦延髓池注射urocortın抑制迷走神經刺激胃運動的角色。計畫二：研究CRF2接受器在小腦延髓池注射urocortin抑制迷走神經刺激胃酸分泌的角色。計畫三：研究人類第二型urocortın經由周

邊CRF2接受器造成全身性低血壓。計畫四：探索胃腸道CRF接受器的表達。

本文電子檔已上傳至出國報告資訊網

摘要（ Abstract ）

Corticotropin releasing factor (CRF)，是由下視丘側腦室旁的神經核所分泌的 41 個胺基酸胜肽，起初是由於它主要能激活下視丘－腦垂體－腎上腺軸（ HPA axis ）對於壓力所產生的反應而有所了解（ Nature 1995; 378: 287-292 ）。 CRF 胜肽家族包含 urocortin（ Endocrinology 1996; 137: 3896 ）和 urocortin II 及 urocortin III (Nat Med 2001; 7: 605-611) (Proc Natl Acad Sci 2001; 98: 2843-2848)。 CRF 及其胜肽家族在心臟血管系統、生殖系統、胃腸道、免疫系統和中樞神經系統方面扮演廣泛的角色（ Nat Genet 2000; 24: 410-414)(Neuron 1998; 20: 1-20 ）。因此， CRF 家族胜肽是科學們探索大腦－胃腸交互作用一個很好的研究物。

CRF 和其相關的胜肽，其生物作用是經由鍵結至他們的接受器而起動，進一步增加細胞內的 cAMP 濃度。至今，兩種 CRF 接受器亞型，CRF_1 和 CRF_2，已經在哺乳動物中成功地分離克隆(clone)出來了（ Mol Endocrinol 1995; 9: 637-645 ）。在我給台灣國家衛生研究院 2000 年的年度結案報告中已經指出，小腦延髓池注射 uroco tin 可經由 CRF 接受器抑制迷走神經所刺激的胃酸分泌和胃收縮。因此，利用我第一年所得到的經驗，我進一步探索 CRF 接受器亞型對中樞調節胃酸分泌和胃運動功能的影響。甚至，使用一個全新 CRF_2 選擇性接受器刺激物：人類第二型 urocortin （ human urocortin II ）去研究週邊 CRF_2 接受器在調節全身血壓的角色。最後，我專注於胃腸 CRF_2 接受器的克隆（ clone ）、特質分析和鑑定。下面就是我出國至 UCLA/CURE 進修兩年的計畫總結，同時這些結果也都寫成論文投到有同儕專家審稿

（ peer-review ）的 SCI 科學期刊：

計畫一：研究 CRF_2 接受器在調節小腦延髓池注射 urocortin 抑制迷走神經刺激胃運動的角色。

計畫二：研究 CRF_2 接受器在小腦延髓池注射 urocortin 抑制迷走神經刺激胃酸分泌的角色。

計畫三：研究人類第二型 urocortin 經由周邊 CRF_2 接受器造成全身性低血壓。

計畫四：探索胃腸道 CRF 接受器的表達。

關鍵字：大腦－胃腸、 CRF : corticotropin releasing factor 、接受器、胃酸、胃運動、低血壓、小腦延髓池、 urocortin 、人類第二型 urocortin 。

台北榮民總醫院內科部胃腸科陳志彥大夫出國進修心得報告書

目次

正文（Test）

壹、背景和目的（Background and Aims）

功能性胃腸問題，包含逆流性食道炎（GERD）、非潰瘍性消化不良（NUD）、大腸急躁症（IBS），甚至包含發炎性腸症（IBD），漸漸取代幽門螺旋桿菌造成之潰瘍和病毒性肝炎，成為二十一世紀世界性的衛生問題。超過百分之二十二的美國人口都被功能性胃腸問題所困擾（Gastroenterology 1993; 38: 1569-1580）；此一議題不可被忽視。不幸地，由於功能性胃腸問題是多因性的，目前仍無一可靠有效的治療方法（Dig Dis Sci 2000; 45: 952-959）。因此，功能性胃腸問題值得科學家們和臨床醫師更多的注意。

絕大多數有功能性胃腸問題的病人，被發現在他們的生活上經歷了較多、較大的壓力（stress）。Corticotropin releasing factor (CRF)，是一個由下視丘側腦室旁神經核所分泌的 41 個胺基酸胜肽，最初是由於它扮演激活下視丘－腦垂體－腎上腺軸（HPA axis）對壓力所產生的反應之角色而被認清（Nature 1995; 378: 287-292）。現今，CRF 和其家族胜肽在心臟血管、生殖系統、胃腸道、免疫系統和中樞神經系統牽涉了愈來愈廣泛的角色（Nat Genet 2000; 24: 410-414）（Neuron 1998; 20: 1-20）。因此，CRF 家族胜肽是科學家們探索大腦－胃腸交互作用很好的研究對象，尤其是壓力造成的胃腸疾病。而發展動物模型是了解這些狀況所必經的步驟。

在大白鼠身上，從小腦延髓池注射（intracisternal injection）壓力性荷爾蒙，例如 CRF 或 urocortin，是研究和解開複雜的大腦－胃腸交互作用迷題的一理想動物模型。首先，在藥理生理實驗上，我們使用

此一小腦延髓池注射壓力性荷爾蒙的動物模型，去決定和了解小腦延髓池注射 urocortin 對抗迷走神經所刺激的胃酸分泌和胃收縮的特質，而胃酸分泌和胃收縮分別代表的是胃黏膜和胃肌肉的功能。第二，我們經由靜脈注射一全新 CRF_2 選擇性接受器刺激物，人類第二型 urocortin ，研究其對系統性血壓的影響。這兩個計畫有助於釐清 CRF 接受器亞型分別在消化系統和心臟血管系統的角色。第三，使用分子生物的方法，我們嘗試在胃腸道上鑑定和克隆出 CRF 接受器之亞型（即 CRF_1 和 CRF_2 接受器），這發現可能可以提供一些線索以回答我們之前的發現。我們期待我們的結果可闡明壓力性荷爾蒙在中樞和周邊的調節作用，並且可以好好解釋人類功能性胃腸疾病的致病機轉。這些努力有可能打開新的研究方式的另一視窗，甚至導致治療功能性胃腸疾病的新的治療藥物的發明。

貳、過程（ Procedure ）和結果（ Results ）

到達美國的第一年，我致力於動物模型的建立。由於是第一次接觸 whole animal study ，花了很多時間去適應和調適，這包含了大白鼠的麻醉、手術的進行、 pH 值的滴定器的校正和使用 strain gauge 晶片的植入置放、胃雙腔管的擺置、電流整流放大器的問題解決、電腦計錄和分析軟體的熟悉等等。其中難度最高的是小腦延髓池留置管（ intracisternal catheter ）和動脈留置管（ intraarterial catheter ）的擺置，稍有不慎，動物便立即死亡，整個實驗即提早宣告失敗。在歷經多次的失敗，累積許多寶貴的經驗，此一動物模型終告成熟穩定，得到高品質的結果。此一成果分別在 American Motility Society 2000 (Neurogastroenterol Motil 2000; 12: 479 ，見附錄一)和 Digestive

Diseases Week 2001 (Gastroenterology 2001; 120 (suppl 1): A533 ，見附錄二) 作了摘要發表，全文則寫成 full manuscript ，投稿於著名的醫學期刊 *European Journal of Pharmacology* 。

有了第一年 whole animal 的經驗，第二年我嘗試新的學習：分子生物（ molecular blology ），這包含組織 mRNA 的萃取、 RT-PCR 、 gel 純化、 ligation 、 transformation 、單株選取、 plasmid DNA miniPrep 、消化之、 DNA 排序等等。很高興的是，使用嶄新的 primers 和 RT-PCR 技術，我們成功地在大白鼠胃腸道克隆（ clone ）出一個全新的 CRF_2 接受器亞型，而此一亞型接受器在上消化道較下消化道為多，且有漸減性的分布。此一發現由分子層次提供了更強而有力的證據，支持 CRF2 亞型接受器在調節胃排空和胃收縮的角色。就文獻查詢，這也是人類第一次在胃腸道分離出 CRF_2 亞型接受器，此一論文正在書寫中， Taché 和 Wu 教授計畫投稿到 *FASEB letters* 。

總之，學習的過程是艱辛的，然而全動物的藥理生理實驗和分子生物奠定了未來研究的基石，其成果是甜美的。

參、心得

美國 UCLA/CURE Taché 教授動物實驗室在「大腦－胃腸軸」的研究的設計、執行和成果都令人耳目一新。該研究中心著重於動物模型的建立，與探索及定位功能性胃腸問題相關的生化傳遞物質，舉凡腦室內注射 CRF ，研究精神性或手術後壓力下 CRF 對胃和大腸運動的改變，在特定腦內神經核注射特殊胜肽抑制胃酸、組織胺與 serotonin 的分泌以探索並定位其在大腦的控制中心，研究決定胜肽作用的神經傳導路徑，評估髓性 TRH 在迷走神經刺激胃功能方面所扮演的生理角

色，以及大腸對機械性刺激之內臟過度敏感的模型建立等等，無不欣欣向榮，領先世界。到 UCLA/CURE 進修，可說是大有收穫。

國內投入基礎醫學的研究的醫師不多，根基也不夠深厚。而臨床醫師大多忙於照顧病人，對於「動物實驗」和「分子生物」十分陌生。缺乏深厚的基礎醫學作為後盾，臨床研究一旦遇到瓶頸，往往不易突破。很高興並且很榮幸得到「國家衛生院」提供的「醫師研究獎勵計畫」，它讓我無經濟和工作上的後顧之憂，前往充滿挑戰性的實驗室接受嚴格的全職的訓練。在此我要感謝內科部李壽東主任和胃腸科張扶陽主任的全力支持，以及台北榮總張院長的玉成。

在我的動物實驗性，計畫一、計畫二得知 CRF_2 亞型接受器在調節小腦延髓池注射 urocortin 抑制迷走神經刺激胃運動、胃酸分泌占一重要角色。計畫三得知人類第二型 urocortin 經由周邊 CRF_2 亞型接受器降低全身性血壓。這些實驗結果，寫成論文摘要，投到美國重要的醫學會議，分別得到 2000 年 American Motility Society 年輕研究員獎（附錄三）和 2001 年 American Society for Gastrointestinal Endoscopy 年輕研究員獎（附錄四）等榮譽，可說是得來不易。在我的分子生物實驗裡（計畫四），在 Dr. Wu 的協助下，我們設計嶄新的 primers，在大白鼠胃腸道分離並克隆（clone）出全新的 CRF_2 亞型接受器，更是為計畫一～計畫三提供強而有力在分子階層的證據。這四個計畫，可說是成功地前後呼應，相互支持。

總之，出國進修，認識世界級大師 Taché 教授，學習和得到新的資訊和技術。在參加醫學學術會議，以文會友，又有另一番收穫。希望返國後，在現有的基礎上繼續努力，回饋於國內的醫藥衛生和生命科學，使人群廣泛受益。

肆、建議（Recommendations）和未來發展計畫（Future Plan）

根據我兩年來在美進修 *in vivo* 和 *in vitro* 實驗的發現，我將繼續努力使用藥理生理、分子生物和組織學免疫染色方法，研究 CRF 接受器亞型在調節感覺（所謂 hyperalgesia）所扮演的角色。這將為回答胃腸至大腦的連繫提供有力的機轉。待完成了解動物 CRF 接受器角色和大腦－胃腸的傳訊（大腦至胃腸或者胃腸至大腦），我希望我能利用動物上的發現去解開非潰瘍性消化不良（ NUD ）病人們的致病機轉的秘密。這些非潰瘍性消化不良病人可進一步區分為胃運動功能不良（胃黏膜分泌過多或過低、胃運動低下和過高）和胃感覺功能不良（ hyperalgesia ）。待我們釐清 CRF 接受器在人類的壓力性胃運動和感覺疾病的角色後，並且與這些病人生活上的壓力事件做關連性，一個嶄新的治療方式可能因而誕生。

另外一個上消化道疾病，逆流性食道炎（ GERD ），也值得我們重視。這些逆流性食道炎病人有下食道括約肌暫時性放鬆的問題，並且此一疾病也與生活壓力有關。利用我們在胃的發現去探索 CRF 接受器在逆流性食道炎的角色就十分有趣。在下消化道疾病中，發炎性腸症（ IBD ）和大腸急躁症（ IBS ）更是兩個與生活壓力有關的疾病；我們更可嘗試利用我們的發現予以進一步的探索。整個消化道與壓力有關的疾病，可以說是我們研究興趣的所在。

回國之後，我希望在分子生物研究上繼續研究 CRF_2 亞型接受器在 cAMP 方面的表達。持續性的努力和堅持是一個人成功必備的特質，也是科學進步最大的動力所在。希望我能持之以恆，回饋於台北榮民總醫院和國家社會。

附錄一

THE EFFECT OF ESOPHAGEAL BODY PERISTALSIS ON ESOPHAGEAL ACID CLEARANCE

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Normal esophageal acid clearance requires a two step process peristalsis rapidly removes the acid volume, and swallowed saliva neutralizes the small amount of residual acid coating on the esophageal mucosa Previous studies have shown that esophageal motility is impaired in patients with reflux esophagitis However, studies that have investigated correlation between esophageal motility and acid clearance are relatively few The aim of present study was to determine the relationship between peristaltic wave parameters (amplitude, duration, and transit time) and esophageal acid clearance Methods Subjects were 29 adults ranging in age from 18 to 84 with a mean age of 51 2 years All individuals had symptoms of heartburn at least four times a week with antacid consumption at least once a week All subjects had an esophageal motility study to evaluate esophageal function The amplitude, duration, and transit time of distal esophageal contractions were measured at 3 cm from manometrically determined upper border of the lower esophageal sphincter (LES) Esophageal pH was measured 5 cm above the proximal border of LES On a separate occasion, the acid clearance test was accomplished The acid clearance process involved the installation of 15 ml of 0 1 NHcl into distal esophagus The patient was required to swallow every 30 seconds, and the number of swallows required to produce an esophageal pH above 4 0 was determined Results There was no relationship between amplitude of peristaltic contractions and number of swallows required for acid clearance (r=0 128) No significant difference was found in number of swallows for acid clearance between the individuals with higher amplitude and these with lower amplitude ($\bar{x}$ higher=9, $\bar{x}$ lower=10, NS) The correlation between duration of peristaltic contractions and number of swallows for acid clearance was also not significant (r=0 016) Conclusions 1) The different esophageal peristaltic wave parameters did not correlate well with acid clearance, suggesting these factors may be important to volume clearance, but not so important to acid neutralization which requires not only effective peristalsis but also salivary secretion 2) Acid clearance is more likely related to failed peristalsis and defective salivary flow

DELAYED GASTRIC EMPTYING IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

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Liver cirrhotic patients sometimes have disturbed gastric emptying (GE) Apparently, there is no study addressing whether patients with hepatocellular carcinoma (HCC) have similar impaired GE Aim Using a homemade impedance tomography to measure liquid GE, we tried to assess the characteristics of GE in HCC patients Methods We enrolled 34 healthy controls and 45 HCC patients to compare their GE After drinking 500 ml of water, 12 electrodes were placed in a circular array around the upper abdomen of studied subjects In a rotating order, paired electrodes injected electrical current and the remaining 10 electrodes recorded signals Based on tomographic calculation, serial changes in averaged signals of altered resistivities were constructed to display GE Patients' demographics, clinical data, and various blood parameters were recorded Results The half-emptying times in controls and HCC patients were 15 14±1 56 (SEM) and 21 38±1 84 minutes, respectively (P<0 05), while the areas under the emptying curve were 1732 2±106 4 and 2246 6±109 8 arbitrary units, respectively (P<0 05) Delayed GE was obvious in HCC, as demonstrated by vomiting and anorexia The cirrhotic component in HCC patients only resulted in a shorter period needed for full distention of the stomach after drinking (4 33±1 02 vs 8 78±2 1 minutes, P<0 05) Other characteristics, including demographics, clinical state, tumor size, ascites, and blood parameters, had no influence on GE Conclusions Liquid GE is delayed in HCC patients, esp in those showing symptoms of vomiting and anorexia

INTRACISTERNAL UROCORTIN INHIBITS RX 77368-STIMULATED GASTRIC ACID SECRETION AND GASTRIC CONTRACTILITY IN URETHANE ANESTHETIZED RATS

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Urocortin (UCN) is a newly characterized mammalian corticotropin releasing factor (CRF)-related peptide, and a potent endogenous ligand for CRF-receptor subtype 2 (Nature 1996, 378 287-292) We previously showed that central CRF inhibited gastric acid secretion (GAS) (Science 1983, 222 935-937) and gastric contractility (GC) (Regul Pept 1988, 21 173-181) However, the effects of UCN on GAS and GC are unknown Aim To investigate the influence of intracisternal (IC) UCN against IC RX77368 (RX, a stable TRH analogue)-stimulated GAS and GC in urethane anesthetized rats Methods GAS was measured by the flush technique through double lumen gastric cannula, and GC was assessed by acutely implanted strain gauge sutured to the serosal side of gastric corpus in 24h fasted male Sprague-Dawley rats (250-300 g) IC injection was performed through acutely implanted IC catheter, and the body temperature was maintained at 37°C IC UCN (3 or 10 μg/rat, 5μl) or IC vehicle (saline, 5μl) was administered 20 min before IC RX (30 ng/rat, 5μl) Results IC injection of UCN (3 or 10 μg/rat) dose-dependently inhibited GAS by 67 6% and 75 9% respectively in comparison with IC vehicle (52 7 ± 22 5, 39 3 ± 11 6 vs 162 8 ± 17 5 μmole/120min, n= 3-5, p<0 05) The duration of RX-induced stimulation of GC was significantly shortened in rats with IC UCN at 10 μg compared to IC vehicle (39 8 ± 11 1 vs 79 8 ± 11 9 min, p<0 05) Conclusion IC UCN inhibits central vagal stimulation of gastric acid secretion and gastric contractility in anesthetized rats

IDENTIFICATION OF THE LIGAND-BINDING DOMAIN OF THE MOTILIN RECEPTOR.

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The human motilin receptor was recently identified (Feighner et al *Science* 1999,284.2184-2188) Based on sequence analysis, it has been classified within a new subgroup of the Class I family of G-protein coupled receptors As this group of receptors was only identified recently, no receptor domains of importance in ligand binding have yet been identified Better understanding of the molecular basis of activation of the motilin receptor by its natural ligand may provide insights useful for drug development. Photoaffinity labeling can provide the means to directly identify the ligand binding domain Methods We, therefore, developed two new radioiodinatable and photolabile agonist ligands of the human motilin receptor that incorporate respectively a p-benzoyl-L-phenylalanine into position Phe^{5} and a p-benzoyl-benzoyl-lysine into position Lys^{20} Both analogues were used to identify spatially approximated receptor domains The ligand binding domain was localized by targeted chemical fragmentation with cyanogen bromide (CNBr) Results The [Bpa^{5},Ile^{13}] human motilin-1-22 and [Lys (Bz-Bz)^{20},Ile^{13}] human motilin-1-22 peptides were full agonists that increased intracellular calcium concentration in a Chinese hamster ovary cell line engineered to express the human motilin receptor They bound specifically and with high affinity (K_i=50 3±7 1 nM and = 2 4±0 2 nM, respectively) to this receptor Both probes covalently labeled the motilin receptor, with the site of attachment within the M_r=45,000 deglycosylated core protein CNBr cleavage of the labeled receptor yielded a major labeled fragment of M_r=17,000, corresponding to the large, second extracellular loop of the motilin receptor Conclusion These data provide the first direct evidence of a molecular approximation between residues within both halves of a motilin-like agonist and a specific domain within the human receptor

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附錄二

5-HT receptors in the regulation of gastro-duodenal MMC activity in man [1]Tack et al, Gut 1998;42 36 [2]Houghton et al, Gastroenterol 1988,94 1276

2710

A Central Nitrergic Pathway Modulates Gastric Motility by Peripheral Endotoxin

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A neural mechanism that involves synthesis of NO in the brain has been shown to inhibit gastric acid secretion under stress (PNAS, 93 14839-14844, 1996) Aim To evaluate the effects of peripheral endotoxin on gastric tone and the pattern of brain neuronal activation induced by 2-deoxy-D-glucose(2-DG) The role of NO has also been evaluated Methods *Intragastric pressure(IgP)* Sprague-Dawley rats were anesthetized and an intraluminal latex balloon inserted in the stomach Endotoxin (40μg/kg,i v) or vehicle (1ml/kg,i v) were administered before 2-DG (200 mg/kg,i v) and IgP was monitored for 60 min Some rats were administered with L-NAME (10mg/kg,i v or 800μg/kg,i c) 15 min before endotoxin *Inmunohistochemistry for c-fos gene* Conscious rats received endotoxin or vehicle 10 min before 2-DG (400mg/kg,i p) and were perfused with fixative, 2 hours later Brain sections were processed for c-fos staining *NOS activity* Conscious rats were sacrified 30 min after administration of endotoxin or vehicle A section of the brainstem (+1 to -1 4 from the obex) was removed and NOS activity measured by the rate of conversion of U-14C-L-arg to U 14C-L-citrulline Data are expressed as mean±SEM Results Data of IgP are included in the table 2-DG increased ($P<0$ 05) the number of FOS positive neurones in the DMN (10±2 3, n=3) and the NTS (75 6±10 7, n=3) when counted bilaterally in 6 sections from +1 to -1 4mm from the obex. Endotoxin reduced ($P<0$ 05) the number of FOS in the DMN (3 3±2 0, n=3) and not in the NTS (40±22 5 n=3) Constitutive NOS activity in the brainstem (expressed as pmol/min/g protein) was increased by endotoxin (11460±1072, n=4), compared with that observed in vehicle-treated rats (7493±262 7, n=4) Conclusion Low doses of peripheral endotoxin which inhibit gastric tone, prevent neuronal activation in the DMN induced by 2-DG suggesting that blockade of the efferent vagal output accounts for the gastric inhibitory effects of endotoxin Central synthesis of NO is involved in this effect

	vehicle, i v	L-NAME, i v	vehicle, i c.	L-NAME i c
saline	1 4±0 5*(10)	2 5±0 8*(6)	1 8±0 4*(10)	2 2±0 7*(9)
endotoxin	-0 6±0 3(10)	1 8±0 4*(8)	0 6±0 3(13)	2 9±0 4*(9)

Results are expressed as the difference between stimulated and basal medium tone (cm H_2O)
*P<0 05 vs the respective endotoxin-treated group

2711

L-Glutamate Mediate Gastric Contraction and Relaxation by Activating Different Subpopulations of Cholinergic Neurons in the Vagal Dorsal Motor Nucleus

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Motor neurons in the dorsal motor nucleus (DMNV) serve to integrate sensory signals of peripheral and central origin and in turn they regulate gastrointestinal activities Recent studies demonstrated that L-glutamate (L-Glu) is present in abundance in the dorsal vagal complex (DVC) Since synaptic activity in rat DMNV neurons are markedly reduced by L-Glu receptor antagonists, this suggests that endogenous L-Glu is a major neurotransmitter controlling activities of DMNV neurons In this study we investigated the effects of L-Glu on gastric motility when microinjected into rat DMNV We first performed immunocytochemistry of GluR 2 receptor and showed that GluR 2 receptor staining was widely distributed in the DVC region Often they were found to colocalize with acetylcholine-containing neurons Depending on the location microinjection of L-Glu (10pmol) in the DMNV produced either gastric contraction or relaxation When L-Glu was injected into the caudal part of DMNV (0 4-0 6mm lateral to the midline at the obex level and 0 6 and 0 6-0 8mm from the dorsal surface of the medulla) it caused gastric relaxation (-1 5 ± 0 2 cm H_2O) This was blocked by hexamethonium (20 mg/kg) and markedly reduced by the VIP antagonist ([p-chloro-D-Phe 6, Leu ^{17}]-VIP) (30 nmol/kg) In contrast intravenous administration of the NOS inhibitor L-NAME (10 mg/kg) had no effect In separate studies we demonstrated that microinjection of L-Glu (10pmol) in the rostral part of DMNV (0 6-0 8mm rostral to the obex, 0 4-0 6mm lateral to the midline at the level of central canal opening) caused gastric contraction (1 4 ± 0 3 cm H_2O) This action was blocked by hexamethonium and atropine (50μg/kg) This indicates that L-Glu stimulates a subpopulation of cholinergic neurons in the caudal half of DMNV and these neurons in turn synapse with intragastric VIP containing neurons to mediate gastric relaxation On the other hand, L-Glu can also activate a different population of cholinergic neurons in the rostral half of DMNV, which synapse with intragastric cholinergic neurons to mediate contraction In conclusion we have demonstrated GluR 2 receptors are present in abundance on cholinergic neurons in the DMNV Depending on the location, these neurons may synapse with intragastric cholinergic neurons or VIP containing neurons to mediate gastric contraction and relaxation respectively

2712

Intracisternal Urocortin Inhibits TRH Analogue-Induced Gastric Contractility Mainly Through Central CRF Receptor 2 In Rats

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BACKGROUND Urocortin (UCN), a new mammalian corticotropin releasing factor (CRF)-related peptide, delays gastric emptying in rats (AJP 1999,276 G867) Intracisternal (IC) CRF inhibits RX 77368 (RX, TRH analogue)-induced vagus nerve dependent stimulated gastric contractility (GC) (Regul Pept 1988,21 173) CRF and its related peptides exert their effects through CRF-R1 and CRF-R2 subtypes However, the receptor subtype through which central UCN exert the inhibitory effect on GC is still unknown AIM To explore receptor subtypes involved in the central modulation of UCN to inhibit RX-induced GC in rats METHODS Under urethane anesthesia, adult male SD rats were acutely implanted with strain gauge sutured to serosal side of gastric corpus to monitor contractions of circular muscles Parameters of GC e g. area under curve (AUC) of contractions, duration of response, and spike frequencies were calculated Gastric acid secretion was assessed by flush technique IC injection was performed through acutely implanted IC catheter IC UCN (3 μg/rat, 5 μl) or saline (5 μl) was given 20 min before IC RX (30 ng/rat, 5 μl) 10 min before IC UCN, the non-selective CRF-R antagonist (astressin 8, ASTB, 30 or 100 μg/rat, 5 μl), selective CRF-R1 antagonist (NBI-27914, NBI, 50 100 or 200 μg/rat, 5 μl), selective CRF-R2 antagonist (antisauvagine-30. AS, 30 or 60 μg/rat, 5 μl) or their vehicles were injected IC or IV RESULTS IC UCN 3 μg decreased AUC of GC induced by RX stimulation (10 01 ± 2.13 vs 22 93 ± 3 03 $p <$ 0 05) IC ASTB 100 μg and IC AS 60 μg antagonized the inhibitory effect of IC UCN on GC (Table 1) CONCLUSIONS IC UCN at 3 μg inhibits RX-induced medullary vagal stimulated GC, as evidence by decreased AUC This inhibitory effect of IC UCN on GC is mediated mainly through central CRF-R2

Table 1 AUC of contractions MEAN ± SE, * $p < 0$ 05 vs Vehicle + RX, n = 4-8

ASTB + UCN + RX	NBI + UCN + RX	AS + UCN + RX
IC 30 μg/rat	IC 50 μg/rat	IC 30 μg/rat
7 32 ± 3 56*	2 80 ± 1 30*	11 66 ± 2 62*
IC 100 μg/rat	IC 100 μg/rat	IC 60 μg/rat
18 38 ± 3 42	4 35 ± 2 31*	16 12 ± 3 06
IV 30 μg/rat	IC 200 μg/rat	IV 100 μg/rat
11 80 ± 5 77*	4 54 ± 2 44*	4 52 ± 3 03*
IV 100 μg/rat		
8 48 ± 1 91*		

2713

Autonomic Nervous System Action In Gastric Hypermotility Induced By Intracisternal TRH Analog, RX 77368, In Anesthetized Rats

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BACKGROUND We showed that intracisternal (ic) RX77368 (RX) induced gastric degranulation of mast cells through sympathetic pathway (Gastroenterology 116.A210, 1999) and that mast cell stabilizers blocked ic RX (2 5, 10 ng)-induced gastric hypermotility in urethane anesthetized rats (Gastroenterology 118 A130, 2000) AIM To investigate the role of the autonomic nervous system in ic RX-induced gastric hypermotility METHODS Intragastric luminal pressure was monitored via a catheter placed into the corpus in urethane anesthetized rats Consecutive ic injections of RX (2 5 and 10 ng) were performed at a 60 min interval with various pretreatments The area of the response (AR cmH_2O*min) and the peak response (PR cmH_2O) were calculated using computer software of WINDAQ Data Acquisition and DADiSP ver 4 1 The basal luminal pressure was maintained at 3 7-4 3 cmH2O (basal area under curve 240 6±2 5 cmH_2O*min basal peak luminal pressure 4 5±0 1 cmH_2O) RESULTS The dose-related increases in AR and PR induced by RX at 2 5 and 10 ng were blocked by vagotomy and reduced by atropine (1 mg/kg sc) by 55-83% (AR) and 75-80% (PR), and bretylium (15 mg/kg sc) by 36-52% (AR) and 62-65% (PR) while propranolol (1 mg/kg iv) had no effect (Table) CONCLUSIONS ic RX-induced gastric hypermotility is primarily mediated by vagal cholinergic stimulation and in part by sympathetic pathway which may be related to gastric mast cell degranulation

(Table) Area of response (AR) and Peak respose (PR) Mean±SEM.

Pretreatment	n	AR RX2 5	AR RX10	PR RX 2.5	PR RX10
Sham ope	7	123 4±25 4	373 3±51 7	8 5±2 8	23 6±5 7
Vagotomy	5	19 6±11 7*	14 0±9 7*	1 1±0 4*	0 8±0 2*
Atropine	6	55 9±22 2	64 6±28 5*	2 1±1 3	4 9±2 2*
Vehicle #1	6	151 3±19 8	376 6±51 7	10 7±2 3	17 3±3
Bretylium	1 0	97 6±19 0	182 0±27 5*	4 1±1 1*	6 1±1 3*
Vehicle #2	6	156 7±51 8	274 7±38 4	10 5±3 3	22 0±5 7
Propranolol	8	138 6±69 0	206 6±52 8	5 7±2 7	7 1±1 8‡

* $p\leq0$ 05 vs Sham ope * $p\leq0$ 05 vs Vehicle #1 and ‡ $p\leq0$ 05 vs Vehicle #2

2714

Role Of Neuronal Nitric Oxide Synthase In Gastric Hypermotility Induced By Intracisternal TRH Analog, RX 77368, In Anesthetized Rats

Koko Kanamoto, CURE/Digestive Diseases Research Ctr, Dept of Med , UCLA, Los Angeles CA Keishi Kawakubo Dept of Medicine and Clin Science, Kyushu Univ, Fukuoka Japan David W Adelson, Yvette Tache, CURE/Digestive Diseases Research Ctr, Dept of Med UCLA, Los Angeles, CA

BACKGROUND Intracisternal (ic) TRH analog, RX77368 (RX), induces vagal atropine-sensitive increase in gastric contractions and nitric oxide (NO) -dependent stimulation of gastric blood flow in rats The interaction between cholinergic transmission and NO in the regulation of gastric contractility has been mainly studied in vitro AIM To assess whether neuronal NO synthase (nNOS) modulates ic RX-induced gastric hypermotility METHODS Intragastric luminal pressure was monitored via a catheter placed into the corpus through the forestomach in anesthetized rats Consecutive ic injections of RX (2 5 and 10 ng) were performed at a 60 min interval The area of the response (AR cmH_2O*min) and the peak response (PR cmH_2O) were calculated using computer software of WINDAQ Data Acquisition and DADiSP ver 4 1 (basal area under curve 238 8±2 0 cmH_2O*min, basal peak luminal pressure 4 7±0 1 cmH_2O) Pretreatment with N_ω-Nitro-L-Arginine methyl ester (L-NAME, 3 mg/kg iv) L-NAME

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This is to certify that

Chih-Yen Chen, M.D.

attended and presented research at the

FIFTH ANNUAL YOUNG INVESTIGATORS'
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P.13

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December 5, 2001

Dr Full-Young Chang, M.D
Chief, Division of Gastroenterology
Taipei Veterans General Hospital
12F, 201, Shih-Pai Road, Sec 2
Taipai, Taiwan (ROC)

Dear Dr Chang,

Dr Chih-Yen Chen is just finishing his two years experimental research training at the UCLA/VA Digestive Diseases Research Center and I would like to thank you for the financial support and time off that you gave to Dr. Chen to work in my laboratories on Brain-Gut Interactions.

During his time at CURE, Dr. Chen acquired expert knowledge in several areas related to brain-gut interactions. This knowledge spanned from conceptual understanding to experimental methods and included surgical, pharmacological, functional and molecular methods of approach. He was extremely dedicated to his research project, working long hours during the week and week ends and completing several projects as follows:

1 He established the role of corticotropin releasing factor (CRF) receptor subtype 2 in the brain medulla as it relates to central inhibition of vagal regulation of gastric motor function His data are presently under review in the *British Journal of Pharmacology*:

Article 1 ***C.H. Chen****, M Million, D W Adelson, V Martinez and Y Taché Intracisternal urocortin inhibits vagally stimulated gastric motility in rats role of* CRF_2 *Brit J Pharmacol Submitted 10/9/01*

2. He provided the first evidence that the novel endogenous ligand for CRF receptor subtype 2, urocortin II characterized in June 2001 is able to prevent the hypertensive response to central sympathetic activation when injected peripherally. His findings were submitted as a rapid communication in the *European Journal of Pharmacology*

Article 2 ***C.H. Chen****, M-L Doong, J Rivier, W Vale and Y Taché Human urocortin II, a novel endogenous CRF ligand inhibits hypertensive response to central sympathetic activation through CRF_2 receptor in rats Eur J Pharmacol. Submitted December 2001*

3. He performed work related to the understanding of ghrelin, a new peptide characterized as the endogenous ligand for the growth hormone secretagogue receptor, established to be released by endocrine cells of the stomach and recently shown to act in the arcuate nucleus to stimulate food intake. He established that ghrelin acts centrally to stimulate gastric acid secretion, motility and blood flow through vagal cholinergic pathways providing new insights into the integration of peptide action to stimulate food intake as well as digestive function. These data are being submitted to the Digestive Diseases Week meeting in 2001 as an abstract and the manuscript is being assembled based on completed experiments.

Article 3 ***C.H. Chen****, K Kanamoto, D St Pierre and Y Taché Intracisternal injection of ghrelin stimulate gastric secretion, motility and blood flow functions through vagal pathways in rats. Am J Physiol to be submitted*

4 While performing experiments on ghrelin, Dr Chen observed that central injection of ghrelin increased blood pressure; his observations were further developed in collaboration with Dr K. Kanamoto who had equipment required to pursue these observations. The manuscript is now being written.

Article 4 K Kanamoto, ***C.Y. Chen*** *and Taché Intracisternal ghrelin increases blood pressure through sympathetic pathways. Neuroreport (to be submitted).*

5 In collaboration with Dr. Vicent Wu, a molecular biologist working with the late Dr. Walsh, he undertook a project to assess the presence of CRF receptor subtype within the gastrointestinal tract, they were able to clone a novel variant of the CRF2 receptor in rats and used new primer to detect the presence of the CRF2 receptor using RTPCR and westernblot The distribution shows a gradient of distribution with higher detection in the upper vs the lower gut. These findings provide strong support, at the molecular levels, for the role of CRF_2 receptors that we previously established to play a role in the regulation of gastric emptying.

Article 5 ***C-Y. Chen****, Y Tache and V Wu Characterization and gut expression of CRF receptors subtype 2 in rats FASEB letters (to be submitted)*

6. Additional studies related to the pharmacologic characterization of CRF subtypes involved in CRF-induced inhibition of gastric acid secretion have also been completed and need to be collated into a manuscript.

Overall, Dr Chen was very diligent and eager to learn and obtained excellent data He presented his work at several national and international meetings throughout these two years including the annual meetings of the American Gastroenterology Association, the 5th International Symposium

on brain gut axis in Oxford, the annual meeting of the American Neuroscience Society in San Diego and the International symposium on Hormones and Receptors in Cairns as shown by the list of his abstracts.

It is obvious that the gathering of all these data required time as well as effort in the learning of new methods of approach, therefore it is expected that during the next year additional demands will be put on Dr. Chen to complete the writing of his results We will work together to have them accepted for publications as soon as possible.

I am grateful for having the opportunity to work with such a focused, dedicated and pleasant fellow as Dr. Chen and thank you for allowing him to study abroad.

Sincerely yours,

Yvette Taché, Ph.D
Professor of Medicine,
UCLA Department of Medicine
Director, CURE Digestive Diseases Research Center
UCLA/VA Greater Los Angeles Healthcare System