CALCITONIN GENE-RELATED PEPTIDE (CGRP) IN COMPLEX BIOLOGY MATRICES: APPLICATION TO PAIN AND INFLAMMATION STUDIES

Pain is a sensation of an unpleasant nature in response to **a noxious stimulus.** Physiologically, pain has a critical function for survival and represents a major evolutionary advantage. Indeed a painful experience can act as **a warning signal for the organism,** thus influencing it to withdraw from a potentially harmful stimulus. One of the main functions of pain is to alert the body to potential injury. **The neural processing of harmful stimuli is called nociception.** Nociceptive pathways involve multiple actors in the peripheral and the nervous system, including but not limited to peripheral afferent nerves, **nociceptors** – peripheral receptors consisting of the free nervous endings of afferent nervous – found widely in the skin and the mucosa, and neuropeptides. Each of these actors constitutes **a putative target to design therapeutics.**

Calcitonin gene-related peptide (CGRP) has been identified as a key vasodilator neuropeptide involved in several pain and inflammation mechanisms. For example, it has been shown that CGRP is released during migraines and that increase in circulating CGRP levels can trigger migraines in patients. To understand the complex mechanisms involved in chronic pain conditions**, it is important to be able to measure the levels of neuropeptides** associated with nociceptive pathways such as CGRP. **Bertin Bioreagent offers ELISA kits for CGRP quantification in complex human and rodent samples.** Today, the Bertin Bioreagent CGRP kits are widely used by scientists and have been extensively cited in pain and inflammation studies.

QUANTIFY CGRP LEVELS IN YOUR SAMPLES USING THE BERTIN BIOREAGENT CGRP ELISA KITS

SUMMARY

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MEASUREMENT OF PLASMA CGRP LEVEL IN PEDIATRIC MIGRAINE PATIENTS – CHIOU & AL. 2019

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CONTEXT

Migraine is a disorder characterized by recurring pulsating headaches with increased sensitivity to light, sound and head movement. It is one of the leading causes of disability.

Few studies have characterized the effect of anti-migraine medications on children. Since most young children cannot accurately describe their symptoms, finding biomarkers that can guide the choice of migraine treatment is crucial.

Calcitonin gene-related peptide (CGRP) has been shown to be a biomarker of migraine in adults. CGRP causes dilatation of both peripheral and cerebral blood vessels. Baseline plasma CGRP levels are significantly higher in migraine adult patients than in healthy controls. Additionally, during a migraine attack, the plasma CGRP levels of both adult and pediatric patients increase and strongly correlate with headache intensity.

In this study, the scientists explore whether CGRP could be a biomarker helping stratification of migrainous children, by studying the relation between plasma CGRP levels and the responses to anti-migraine therapies.

MATERIALS

- **CGRP (human) ELISA kit** (Cat No. A05481, Bertin Bioreagent, France).
- **Plasma samples** stored at −80°C between sampling and analysis

PROTOCOL

- 120 pediatric subjects were recruited, who never took anti-migraine drugs, including 68 patients with migraine (M), 30 with non-migraine headache (H), and 22 non-headache controls (C).
- Blood (3 ml) sampled from the cubital vein of each patient was put into a 5 ml Lavender tube (BD vacutainer™, Becton Dickenson, Plymouth, UK).
- **Plasma samples** were prepared by centrifugation (2,000 rpm for 15 min) of the whole blood and then stored at −80°C.
- **Plasma CGRP levels** were measured using **CGRP (human) ELISA kit** (Cat No. A05481, Bertin Bioreagent, France). The person who conducted the CGRP measurement was blinded to the identity, attack status, and treatment of the study patients.
- Short-term therapeutic response to anti-migraine drugs was measured for at least 2 weeks after the start of therapy. Responders were defined with >50% headache reduction.

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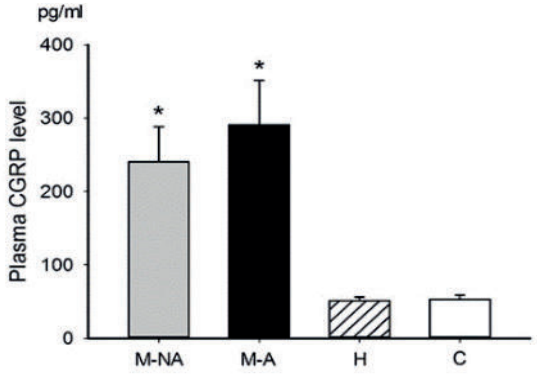
Department of Pediatrics, National Taiwan University Hospital, Taipei, Taiwan

RESULTS

Mean plasma CGRP level is found higher in migraineurs compared to non-migraine headache patients and to non-headache patients. This higher plasma CGRP is observed during migraine attack as well as between attacks.

Topiramate responders had higher plasma CGRP levels than non-responders (437 ± 131 pg/ml, n = 14 vs. 67 ± 19 pg/ml,

n = 6, p = 0.021). Survival curves of plasma CGRP levels also showed those with higher CGRP levels responded better to topiramate



Plasma CGRP levels in different groups.
*The mean plasma CGRP levels in the migraineurs either during (group M-A) or between (group M-NA) attacks were higher than the non-migraine headache patients (group H) (p = 0.006 and 0.018, respectively) and non-headache controls (group C) (p = 0.016 and 0.045, respectively). The p-value among groups, analyzed by ANOVA test (p = 0.001), followed by post-hoc Tukey HSD. *p < 0.05*

Anti-migraine drug	CGRP in pg/ml (*n*)		*p*-value§
	Responders	Non-responders	
Cyproheptadine	191 ± 52 (4)	473 ± 188 (8)	0.45
Flunarizine	243 ± 77 (17)	466 ± 158 (13)	0.219
Topiramate	437 ± 131 (14)	67 ± 19 (6)	0.021*
Gabapentin	421 ± 253 (6)	352 ± 265 (6)	0.522

*Comparison of plasma CGRP levels between responder and non-responder to anti-migraine drugs. Data are mean ± SE with the n number in parentheses. The p-value between responder and non-responder groups, analyzed by Mann-Whitney test * p<0.05.*

CONCLUSION

- This study suggests that the plasma CGRP could be a potential biomarker to help differentiating migraine from non-migraine headache condition in pediatric patients. In this study, the CGRP plasma levels were found considerably higher in topiramate-responsive children indicating that CGRP could also be investigated as an biomarker to categorize pediatric migraine patients. These findings are especially important for young children who cannot precisely describe their symptoms .
- The CGRP ELISA kit from Bertin Bioreagent allows accurate and reliable measurement of CGRP levels in human plasma samples.

Fan PC, Kuo PH, Lee MT, Chang SH, Chiou LC.
Plasma Calcitonin Gene-Related Peptide: A Potential Biomarker for Diagnosis and Therapeutic Responses in Pediatric Migraine.
Front Neurol. 2019 Jan 24;10:10. doi:10.3389/fneur.2019.00010.

EVALUATION OF CIGUATOXIN-INDUCED CGRP RELEASE IN MOUSE AND RAT SKIN PREPARATIONS

QUANTIFY CGRP LEVELS IN RODENT SKIN PREPARATIONS USING THE BERTIN BIOREAGENT CGRP ELISA KITS

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CONTEXT

Ciguatoxins are a group of toxins that are present in certain fish and are responsible for a type of severe food poisoning called ciguatera. The consumption of tropical and subtropical fin fishes contaminated by ciguatoxins can cause several painful neurological, gastrointestinal, and cardiovascular symptoms. Ciguatoxins are known to be powerful voltage-gated sodium channel activator toxins, which partially explains some of the sensory symptoms associated with ciguatera. However, for many of the neurological symptoms caused by ciguatoxins, the underlying biological mechanisms remain poorly understood. It has been recently shown that ciguatoxins are extremely effective at releasing calcitonin-gene related peptide (CGRP) from nerve terminals. **(1)**. Here, we use the **CGRP ELISA kit** to evaluate the release of CGRP in mouse and rat skin preparations induced by Pacific Ciguatoxin-1 (P-CTX-1).

MATERIALS AND PROTOCOLS

- **Skin preparations:** The skin flaps of the lower hindpaws of young male Wistar rats (70–80 g; n = 39) and male C57BL/6J mice (15–35 g; n = 136) were used for the release experiments. The preparation of the skin started at knee level and excluded the toes and it spared larger vessels, and saphenous and peroneal nerve stems. The obtained murine skin flaps had an average weight of 0.10 g while the average weight of rat skin flaps was 0.28 g. They were wrapped around acrylic glass rods with the corium side exposed to the surrounding solution and were fixed with surgical silk threads. The mounted skin flaps were then washed for 30 min in carbogen-gassed synthetic interstitial fluid (SIF, pH 7.4) whose composition is described in **(1).**

- **Stimulation procedures**: After the initial 30 min wash-out in SIF, the skin flaps were placed for 5 min each into pre-warmed glass tubes mounted in a shaking bath at 32 °C. One day prior to the experiments, the tubes were treated with Sigmacote© (Sigma-Aldrich, Taufkirchen, Germany) to prevent adhesion of CGRP and P-CTX-1 to the glass surface. Sigmacote® was refreshed after each 10 experiments. All tubes were filled with 0.7 mL of SIF or stimulation solution containing various concentrations of P-CTX-1 or drugs

- **ELISA assays**: iCGRP (immunoreactive calcitonin gene-related peptide) content in the eluates was measured using the CGRP rat ELISA kit (A05482, Bertin Bioreagent, France) as described in (2,3). Briefly, the samples were mixed with 5-fold concentrated commercial CGRP enzyme immunoassay buffer (200 µL sample + 50 µL buffer. The enzyme immunoassays were run on 96 well plates, which were photometrically determined using a microplate reader (Dynatech, Channel Islands, UK). The minimum detection limit observed was 5 pg/mL. The intra and inter-assay coefficients of variation with repeated measurements were 10–15%. Results can be seen in Figure 1.

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EVALUATION OF CIGUATOXIN-INDUCED CGRP RELEASE IN MOUSE AND RAT SKIN PREPARATIONS

RESULTS

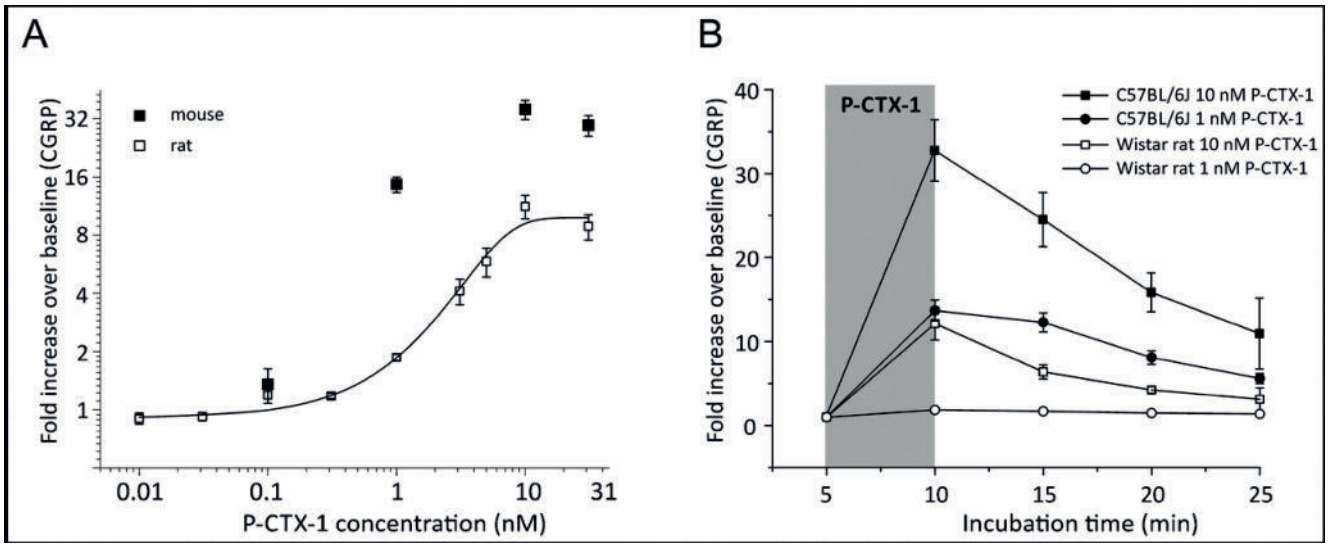

Figure 1: CGRP-release by P-CTX-1 is more effective in mouse than in rat skin. From (2).

(A) Increasing concentrations of P-CTX-1 augmented the iCGRP release dose-dependently in rat and mouse hindpaw skin. The diagram illustrates fold increase of CGRP as compared to baseline. Error bars represent ± SEM; n = 4–18 (see text).

(B) (B) Release of iCGRP following exposure of rat or mouse hindpaw skin with P-CTX-1 at 1 or 10 nM. Stimulation in mouse tissue was more effective and iCGRP release remained high in the incubation steps after P-CTX-1 was removed. Data are measured as pg/mL and expressed as fold increase over baseline.

CONCLUSION

The results of this study show that P-CTX-1 (Pacific Ciguatoxin-1) is more effective at inducing CGRP release in mouse skin as compared to rat skin, with EC50 concentrations in mouse skin preparations in the low nanomolar range. Since CGRP plays a major role in pain and itch sensing pathways, these findings can help further our understanding of the neurosensorial symptoms of ciguatera, and guide the identification of potential therapeutic targets. The **CGRP Rat ELISA kit** from Bertin Bioreagent allows accurate measurements of CGRP in mouse and rat skin preparations.

Universitätsklinikum Erlangen

1. Touska, Filip, et al. "Ciguatoxins evoke potent CGRP release by activation of voltage-gated sodium channel subtypes Nav1. 9, NaV1. 7 and NaV1. 1." Marine drugs 15.9 (2017): 269.
2. Zimmermann, K., P. W. Reeh, and B. Averbeck. "S (+)-flurbiprofen but not 5-HT1 agonists suppress basal and stimulated CGRP and PGE2 release from isolated rat dura mater." Pain 103.3 (2003): 313-320.
3. Averbeck, B., and P. W. Reeh. "Interactions of inflammatory mediators stimulating release of calcitonin gene-related peptide, substance P and prostaglandin E2 from isolated rat skin." Neuropharmacology 40.3 (2001): 416-423.

MEASUREMENT OF CGRP RELEASE FROM MOUSE SAPHENOUS AND VAGUS NERVES FOLLOWING CHEMICAL AND ELECTRICAL STIMULATION

EVALUATE CGRP RELEASE IN MOUSE NERVES USING THE BERTIN BIOREAGENT CGRP ELISA KITS

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CONTEXT

Cutaneous nerves and visceral nerves are peripheral sensorial nerves that possess two distinct functions: cutaneous afferent nerves carry information from the body's external environment, while visceral nerves carry information from the body's internal environment. As cutaneous and visceral nerves have different physiological roles, it has been suggested that they also exhibit different conductive properties.. In this work, this hypothesis is investigated using the saphenous nerve originating from the lumbar root ganglia, and the vagus nerve arising from the nodosum ganglia as examples for cutaneous and visceral nerves. To compare the conductive properties of the saphenous nerve and the vagus nerve, the CGRP release in these nerves is evaluated in response to chemical and electrical stimulation, with the CGRP Rat ELISA kit (Bertin Bioreagent, France).

MATERIALS AND PROTOCOLS

- **Preparation of mouse saphenous and vagus nerves:** The saphenous nerves were dissected bilaterally from their point of leaving the inguinal region to a distance of around 5 mm under the knee. Vagus nerves were exposed and cut from the bifurcation point at heart level to their stomach innervation. The nerve sheaths were removed (both the epineurium and perineurium).

- **Stimulation procedures**: The nerves were placed in a specialized recording chamber (Avere Solutions UG (former Axolent)) between two glass tubes, modified as described in (1). The bath was perfused with SIF (Synthetic interstitial fluid), bubbled continuously with carbogen , and temperature-regulated with a Peltier thermode, continuously throughout the experiment duration. For the electrical stimulation, the cut ends of the nerve were placed through a silicon membrane of the glass tubes. Silver wire electrodes in one of the glass tubes were used as the cathode and anode. The electrical stimulation protocol consisted of stimuli of 0.25 Hz, 3/6/9 Hz, and 0.25 Hz again (for recovery). The chemical stimulation consisted of the application of a 60 mM depolarizing potassium solution (prepared as described in ref) and a solution of 0.3 mM capsaicin (Sigma Aldrich, Germany) diluted in SIF. Each stimulus was only given once to reduce the desensitization of the nerves. The duration of the stimuli was 5min with a 5min pause between each stimuli for recovery.

- **ELISA assays**: CGRP levels during the electrical stimulation and chemical stimulation experiments were evaluated with the **CGRP rat ELISA kit** (A05482, Bertin Bioreagent, France) as described in (2). Briefly, the eluates of the CGRP release experiment were mixed 4:1 with 5-fold concentrated commercial CGRP–EIA buffer. The CGRP–EIA had to be run immediately after the release experiment to prevent the loss of neuropeptide. Because of the low levels of CGRP in the vagus nerve, CGRP release from the vagus (but not saphenous) nerve was measured from two nerves threaded together into the recording and stimulating suction electrodes, to obtain CGRP levels above the ELISA kit's detection threshold. The CGRP release results were divided by two in order to have the per-nerve release. The ELISA assays were un on 96 well-plates, which were photometrically determined using a microplate reader (Opsys MRTM, Dynex Technologies, Chantilly, VA). Results can be seen in **Figure 1**.

MEASUREMENT OF CGRP RELEASE FROM MOUSE SAPHENOUS AND VAGUS NERVES FOLLOWING CHEMICAL AND ELECTRICAL STIMULATION

RESULTS

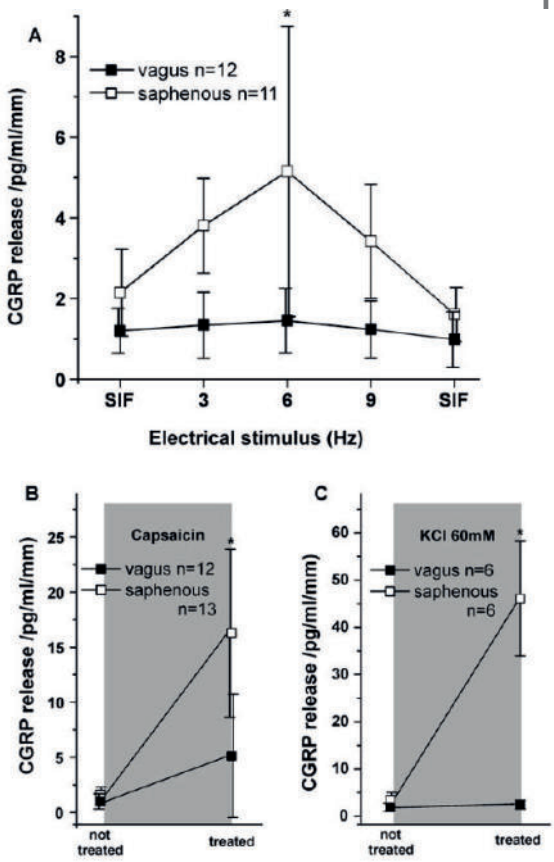

Figure 1: CGRP release from vagus and saphenous nerves. (From (1).)

The CGRP release is quantified as picogram neuropeptide per milliliter fluid released per millimeter nerve.

(a) CGRP release during electrical stimulation at different frequencies (3, 6, and 9 Hz). Saphenous (white squares) release is significantly increased with a maximum at 6 Hz. For the vagus nerve, only a minor nonsignificant increase is observed.

(b, c) Capsaicin (0.3 mM)- and KCl (60 mM)-induced release in saphenous (white squares) versus vagus (black squares) nerves. Capsaicin significantly increased CGRP release for the saphenous nerve but not for the vagus nerve. With the KCl depolarizing solution, a significant increase in the CGRP release was only observed in the saphenous nerve.

CONCLUSION

The findings of this study indicate that both electrical and chemical stimulation can induce CGRP release in unsheathed segments of saphenous nerves, but not in unsheathed segments of vagus nerves. These results, together with measurements of conduction velocity changes, show that vagus and saphenous nerves possess different activation and conduction properties, which could correlate with their distinct physiological roles. A better understanding of the mechanisms that modulate these conduction properties could help further our understanding of pathologies associated with cutaneous and visceral afferent nerves. The CGRP Rat ELISA kit from Bertin Bioreagent allows accurate measurements of CGRP in mouse nerves.

1. De Col, Roberto, Karl Messlinger, and Tali Hoffmann. "Differential conduction and CGRP release in visceral versus cutaneous peripheral nerves in the mouse." Journal of neuroscience research 96.8 (2018): 1398-1405.
2. Averbeck, B., P. W. Reeh, and M. Michaelis. "Modulation of CGRP and PGE2 release from isolated rat skin by α-adrenoceptors and κ-opioid-receptors." Neuroreport 12.10 (2001): 2097-2100.

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