EDUCATIONAL RESEARCH IN ACTION

Utilizing Molecular Details of the Pain System to Illustrate Biochemical Principles

Robert Boal, PhD, **Richard G. Gillette**, PhD, and **William H. Borman**, PhD,
University of Western States

To capture student interest and show clinical relevance, molecular details from the pain system can be used as supplemental examples to basic biochemistry lectures. Lecture topics include glutamate, substance P, calmodulin-dependent protein kinase II, synaptic proteases, calcitonin gene-related peptide, and neuronal protein synthesis. These topics are utilized to illustrate basic biochemical issues and are linked to pain-related topics such as pain transmission, synaptic plasticity, long-term potentiation, and central sensitization. For analysis, a brief survey was administered to evaluate student attitudes toward a representative lecture segment. Survey results support the premise that utilizing the pain system is an effective tool to engage chiropractic students during basic biochemistry lectures. (J Chiropr Educ 2010;24(2):187–193)

Key Indexing Terms: Biochemistry; Chiropractic; Education; Neuronal Plasticity; Pain

INTRODUCTION

Teaching biochemistry in any setting is challenging. As part of the onslaught of 1st-year basic science instruction at a chiropractic college, teaching biochemistry becomes a truly difficult endeavor. Typically, the various anatomical science courses are the focus of beginning chiropractic students, so the biochemistry instructor must resort to any and all strategies to catch the students' attention. One approach to engage students in any basic science course is to utilize clinical correlations.[1–5] This works well in biochemistry, to an extent; some good examples are bilirubin, alkaline phosphatase, and serum lipids, among others. There are, however, limitations and trade-offs with this approach (ie, many clinically relevant examples are obscure and patients with these conditions are less likely to present in a chiropractor's office).

The Journal of Chiropractic Education

Vol. 24, No. 2. Printed in U.S.A.
1042-5055/$22.00

A clinical complaint guaranteed to be encountered in any chiropractic practice is pain. With recent advances in understanding the pain system at the cellular and molecular levels,[6–9] pain represents a terrific instructional tool to illustrate basic biochemical concepts. Biochemistry at the University of Western States (UWS) is taught as a two-quarter sequence. The first course covers fundamentals with the focus on amino acid and protein structures, enzymes, and nucleic acids (including transcription and translation). The second course deals with intermediary metabolism and the roles of vitamins. Students are typically more engaged in the topics covered in metabolism and vitamins due to inherent nutritional connections, so molecular details from the pain system are primarily utilized to accent the more basic biochemical concepts in the first biochemistry class. What follows is an example utilizing glutamate that illustrates a method for introducing pain topics into basic biochemistry lectures. Additionally, other pain-related topics are provided with accompanying background references. Results are presented from a brief survey of student attitudes toward this lecture approach.

METHODS

Lecture Topics

A list of pain-related topics utilized in the introductory biochemistry course taught at UWS is displayed in Table 1. The sequence of topics in the table follows that of pain-related presentations in the UWS biochemistry course. This lecture approach involves augmenting basic biochemical concepts with brief lecture segments, typically lasting 15 minutes, which utilize the cellular and molecular events during pain transmission. The primary focus is on events or activities associated with wide dynamic range (WDR) neurons, the primary pain transmission cell in the dorsal horn of the spinal cord. As a means to illustrate this approach, a lecture segment involving glutamate and its role in pain signaling is presented below. Additional pain-related topics utilized throughout the biochemistry course are described briefly.

Glutamate is the primary excitatory neurotransmitter in the central nervous system (CNS) and plays a critical role in transmitting sensory input derived from noxious stimuli.[10,11] To set the stage for this discussion of glutamate, as well as other pain topics, a brief depiction of a classic pain study[12] is presented at the beginning of the lecture segment (ie, the "rodent paw-clamp model"). In the experimental model described, a rodent is subjected to

Table 1. Potential biochemical topics to be correlated to the pain system

Topic	Connections to Pain System	References
Glutamate		
	• CNS neurotransmitter	10, 11
	• Nociceptors	12
	• NMDA R/Cs	10
	• AMPA R/Cs	10, 13
	• WDR neurons	6, 12, 23
	• Synaptic plasticity	14–18
	• Long-term potentiation	16–18, 20, 21
	• Central sensitization	7, 20, 22, 23
Substance P		
	• Neurotransmitter co-release	26, 27
	• Neurokinin R/Cs	24, 25
	• Synaptic plasticity	14–18
	• Long-term potentiation	16–18, 20, 21
	• Chronic pain	21
CaMKII		
	• Synaptic plasticity	19
	• Long-term potentiation	14, 19, 20, 22
Synaptic proteases		
	• Actions	28, 29
	• CGRP	32–34
	• Substance P	33, 34
RNA splicing		
	• CGRP	32
	• Substance P	33–35
Transcriptional control		
	• WDR neurons	6, 12, 23
	• *c-fos*	12, 36–38
	• Fos	37, 39, 40
	• Ca^{2+}	12, 19, 38
	• cAMP	12, 38
Protein synthesis		
	• Localized dendritic protein synthesis	41–43
	• Synaptic plasticity	41, 42

acute, repetitive noxious stimuli by the attachment of a clamp to the animal's paw. Whether using chemical algogens (eg, capsaicin) or mechanical noxious insult to an animal, this type of experimental approach[6] has led to much of the understanding of the cellular and molecular events in neurons responding to acute noxious stimuli.[6–9] The rodent paw-clamp model gives the student a simple (but memorable) visual model of the pain system and it is referred to throughout the term. The rodent paw-clamp model is only described during the lecture segment; however, as a symbolic act of sympathy for the animal and as a means to grab the students' attention, this portion of the lecture is given while a large paper clamp is affixed to several digits of the lecturer's hand. Warning!!! During the course of the discussion the clamp can become quite uncomfortable. The rodent paw-clamp experimental pain model conveniently leads to discussion of glutamate release from afferent nociceptors, WDR neurons in the dorsal horn, glutamate receptors [*N*-methyl-D-aspartate receptors (NMDA-Rs)[10] and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPA-Rs)[10,13]], synaptic plasticity[9,14–16], long-term potentiation (LTP),[17–21] and central sensitization.[7,20,22,23] These descriptions are brief (due to the presence of the paper clamp and its progressive impact on the lecturer's focus) and rudimentary (as these students are in the first term at UWS). Most important, the students are warned that the term central sensitization is becoming widely employed and its exact meaning may depend on the user and context.[22] The International Association for the Study of Pain (IASP) uses the definition, "an enhanced responsiveness of nociceptive neurons in the CNS to their normal afferent input." Other references to central sensitization may imply a heightened awareness of pain perception without clarifying where or how the pain is generated.[22] The idea of plasticity and, more specifically, synaptic plasticity is further discussed and its historical context is stressed.[20]

Other pain-related topics (Table 1) that lend themselves to basic biochemistry lecture examples include substance P,[24–27] calmodulin-dependent protein kinase II (CaMKII),[19] synaptic proteases,[28,29] calcitonin gene-related peptide (CGRP),[30–35], *c-fos*/Fos,[6,12,36–40] and neuronal protein synthesis.[41–43] For example, the 11 amino acid peptide substance P is co-released with glutamate and its role in nociception is emphasized. CaMKII is discussed as a specific example of a kinase whose involvement in neuronal plasticity is pivotal. Synaptic proteases are presented as a special subset of the large family of proteases which, among their many actions, degrade peptide neurotransmitters. CGRP and calcitonin demonstrate an excellent example of alternative RNA splicing. Both these peptides are physiologically important and CGRP is linked to substance P and nociception. Activation of *c-fos*, an immediate early gene, is discussed as one of the early events in WDR neurons during pain transmission. Its protein product, Fos, acts as a transcription factor and serves as a dramatic example of regulating protein synthesis at the transcriptional level. Finally, localized dendritic protein synthesis is contrasted to axonal protein synthesis and discussed in the context of neuronal plasticity.

Analysis

As a measure of students' reactions to this lecture approach, a brief questionnaire (see Appendix) was administered to a large student group ($n = 89$) who took biochemistry at UWS in fall 2008. The survey focused on the lecture segment involving the amino acid glutamate and its role in pain transmission in the dorsal horn. This glutamate segment was selected because it introduced the overall approach of using pain topics in the biochemistry lectures and described an experimental model to be used in this and subsequent pain-related segments of the course. The questionnaire was approved by the UWS Institutional Review Board as an expedited review. The survey was administered 6 months after the students had received the glutamate lecture and was given in a classroom setting to encourage a high return rate. Students participated anonymously and were blinded to the potential use of the questionnaire results. The survey was independently collected and tallied. Student responses were based on a 5-point scale.

RESULTS

The outcomes from the student survey are presented in Table 2. The overall positive responses support the basic premise that utilizing the pain system is an effective method to engage chiropractic students during basic biochemistry lectures. The positive responses support both the use of an experimental pain model in the presentation and attempts by the instructor to do something illustrative (ie, paper clamp attached to lecturer's digits during the lecture). The response rate for the 6-month sampling

Table 2. Results from fall 2008 presentation

Survey Item	Yes	No
Prior to this lecture, were you aware that the amino acid glutamate was a neurotransmitter?	33	51

Survey Item	Disagree	Slightly Disagree	Neutral	Slightly Agree	Agree	Average
In general, illustrating basic science concepts by utilizing practical and clinical applications is something I appreciate.	0	0	4	9	71	4.8
The lecture segment describing glutamate as a neurotransmitter in the pain system effectively caught my attention.	0	0	8	30	46	4.5
Topic of pain was an important factor in capturing my interest regarding glutamate.	0	0	11	23	50	4.5
The use of a simple experimental model (rodent with paw clamp) was a useful tool to give context to glutamate's role as a neurotransmitter.	0	1	6	21	56	4.6
Attaching a paper clamp to the instructor's fingers (simulating the experimental model) was useful in making the presentation memorable.	0	1	3	11	69	4.8

Note: 84/89 Respondents. Survey taken 6 months postlecture. 5-point scale (disagree-1, slightly disagree-2, neutral-3, slightly agree-4, agree-5).

group was 94% and demonstrates that the survey method provided representative sampling.

DISCUSSION

This paper describes an instructional approach for teaching basic biochemistry that correlates well with chiropractic training. In addition to making biochemical instruction more pertinent for chiropractic students, this approach provides early exposure to the dynamic nature of the nervous system and fundamental issues regarding the processing of noxious stimuli. However, there are pitfalls with this lecture approach. Adding auxiliary topics to an already packed course schedule may be problematic. Also, there is the possibility that first-quarter chiropractic students may be overwhelmed by this advanced subject material. For both these reasons, the discussion of the pain system in these lecture segments is very basic and no attempt is made to cover pain transmission exhaustively. Only the basic molecules and general events are stressed. Concepts such as neuronal plasticity, long-term potentiation, and central sensitization are only introduced and left to be addressed more thoroughly in subsequent courses.

The rodent paw-clamp model is used repeatedly in pain-related lecture segments throughout the course.

Repeated use of the same memorable experimental model expedites the delivery of information and minimizes the impact on coverage of other course topics.

The student survey was designed to elicit feedback on the general impression of the first pain-related lecture segment involving the amino acid glutamate. While the results were positive, they only provide qualitative feedback regarding student impressions. No attempt was made in this study to assess the quantitative impact (ie, student retention of the specific content discussed in the glutamate lecture). Also, feedback on additional lecture segments was not surveyed, so it is uncertain whether subsequent topics would produce equally favorable survey results. The questionnaire did address key issues pertinent to this lecture approach, and timing the survey at 6 months postlecture provides evidence that this lecture segment and overall instructional method were memorable and captured student interest. Given the volume and intensity of basic science coursework in 1st-year chiropractic programs, this is a significant outcome.

A reasonable critique of the student questionnaire is the use of descriptive prompts in the survey introduction. Given the 6-month interim between the lecture segment and the analysis as well as the brevity of the glutamate segment (20 minutes) nested within the numerous 1st-year basic science lectures, the authors felt it was reasonable to remind the students of the lecture segment without influencing their qualitative responses.

This questionnaire was created without pilot testing and no attempt has been made toward validity testing of the questionnaire results. In any follow-up survey, recall bias can be a factor and its impact on responses can be difficult to predict.[44] Relatedly, the overall success of the students in the biochemistry course may also factor into the survey responses and represent another internal validity issue.

By their very nature, these pain-related topics link biochemistry lectures with other classes in the chiropractic program. The instructor frequently reminds students of related issues covered concurrently in cell biology and suggests how a subsequent neurophysiology course will elaborate on a given topic.

The accompanying references (Table 1) were selected on the basis of providing background material for an instructor (without extensive training in the neurosciences) interested in incorporating pain-related topics in a basic science course. No attempt was made to catalog an exhaustive list of primary articles; however, those can be found in a number of the reviews cited below. The references include a limited number of primary sources where historical relevance was considered critical, numerous review articles because the authors believed that those would be the most useful tools for the reader, and a few tertiary sources when more background information was deemed useful.

CONCLUSION

Various examples of links between basic biochemical issues and pain topics are provided. Student survey results demonstrate that utilizing pain-related topics to augment fundamental biochemical concepts can be an effective instructional method in a chiropractic degree program. Pertinent references are cited as tools for those instructors interested in adopting these auxiliary topics into a basic biochemistry course.

FUNDING SOURCES AND CONFLICTS OF INTEREST

There are no funding sources or conflicts of interest to declare.

About the Authors

Robert Boal, Richard Gillette, and William Borman are all with the Basic Science Division, University of Western States. Address correspondence to: Robert Boal, University of Western States, 2900 NE 132nd Avenue, Portland, OR 97230 (e-mail: bboal@uws.edu). This article was received February 7, 2009, revised June 4, 2009 and January 14, 2010, and accepted March 6, 2010.

REFERENCES

1. Neame RLB. The preclinical course of study: help or hindrance. J Med Educ 1984;59:699–707.
2. Schmidt H. Integrating the teaching of basic sciences, clinical sciences, and biopsychosocial issues. Acad Med 1998;73(Suppl 9):S24–S31.
3. Koens F, Mann KV, Custers EJFM, Ten Cate OTJ. Analysing the concept of context in medical education. Med Educ 2005;39:1243–9.
4. Woods NN. Science is fundamental: the role of biomedical knowledge in clinical reasoning. Med Educ 2007;41 (2):1173–7.
5. Vadivelu J. Evaluation of basic science knowledge retention in clinical teaching. Med Educ 2008;42(5):520–1.
6. Dubner R, Basbaum AI. Spinal dorsal horn plasticity following tissue or nerve injury. In: Melzack R, Wall

PD, ed. Textbook of pain, 3rd ed. London: Longman Group UK Limited; 1994, pp. 225–41.
7. Coderre TJ, Katz J, Vaccarino AL, Melzack R. Contribution of central neuroplasticity to pathological pain: review of clinical and experimental evidence. Pain 1993;52:259–85.
8. Costigan M, Woolf CJ. Pain: molecular mechanisms. J Pain 2000;1(Suppl 1):35–44.
9. Sheng M, Kim MJ. Postsynaptic signaling and plasticity mechanisms. Science 2002;298:776–80.
10. Hassel B, Dingledine R. Glutamate. In: Siegel GJ, Albers RW, Brady ST, Price DL, eds. Basic neurochemistry, molecular, cellular, and medical aspects, 7th ed. Burlington, MA: Elsevier Academic Press; 2006, pp. 267–90.
11. Cooper JR, Bloom FE, Roth RH. The biochemical basis of neuropharmacology, 8th ed. New York: Oxford University Press; 2003, pp. 132–50.
12. Bullitt E. Somatotopy of spinal nociceptive processing. J Comp Neurol 1991;312:279–90.
13. Derkach VA, Oh MC, Guire ES, Soderling TR. Regulatory mechanisms of AMPA receptors in synaptic plasticity. Nat Rev Neurosci 2007;8:101–13.
14. Boal RW, Gillette RG. Central neuronal plasticity, low back pain and spinal manipulative therapy. J Manipulative Physiol Ther 2004;27:314–26.
15. Woolf CJ, Salter MW. Neuronal plasticity: increasing the gain in pain. Science 2000;288:1765–8.
16. Bear MF, Malenka RC. Synaptic plasticity: LTP and LTD. Curr Opin Neurobiol 1994;4:389–99.
17. Bliss TVP, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 1993;361:31–39.
18. Sanes JR, Lichtman JW. Can molecules explain long-term potentiation. Nat Neurosci 1999;2:597–604.
19. Lisman J, Schulman H, Cline H. The molecular basis of CaMKII function in synaptic and behavioural memory. Nat Rev Neurosci 2002;3:175–90.
20. Sandkuhler J. Learning and memory in pain pathways. Pain 2000;88:113–8.
21. Pocket S. Spinal cord synaptic plasticity and chronic pain. Anesth Analg 1995;80:173–9.
22. Sandkuhler J. Understanding LTP in pain pathways. Molecular Pain 2007;3:9.
23. Woolf CJ, Salter MW. Plasticity and pain: role of the dorsal horn. In: McMahon SB, Koltzenburg M, eds. Wall and Melzack's textbook of pain, 5th ed. Philadelphia: Elsevier; 2006, pp. 92–105.
24. Trafton JA, Basbaum AI. The contribution of spinal cord neurokinin-1 receptor signaling to pain. J Pain 2000;1(Suppl 1):57–65.
25. Cooper JR, Bloom FE, Roth RH. The biochemical basis of neuropharmacology, 8th ed. New York: Oxford University Press; 2003, pp. 331–34.
26. Kandel ER, Schwartz JH, Jessel TM. Essentials of neural science and behavior. Norwalk, CT: Appleton & Lange; 1995, pp. 293–306.
27. Mains RE, Eipper BA. Peptides. In: Siegel GJ, Albers RW, Brady ST, Price DL, eds. Basic neurochemistry, molecular, cellular, and medical aspects, 7th ed. Burlington MA: Elsevier Academic Press; 2006, pp. 317–32.
28. Frischknect R, Fejtova A, Viesti M, Stephan A, Sonderegger P. Activity-induced synaptic capture and exocytosis of the neuronal serine protease neurotrypsin. J Neurosci 2008;28:568–79.
29. Nakanishi H. Proteases and synaptic activity. In: Lendeckel U, Hooper NM, ed. Proteases in the brain. New York: Springer; 2005, pp. 303–22.
30. Lewin B. Genes IX. Sudbury, MA: Jones and Bartlett Publishers; 2008, pp. 37–97, 667–705.
31. Li Q, Lee J, Black DL. Neuronal regulation of alternative pre-mRNA splicing. Nat Rev Neurosci 2007;8: 819–31.
32. Lou H, Gagel RF. Alternative RNA processing-its role in regulating expression of calcitonin/calcitonin gene-related peptide. J Endocrinol 1998;156:401–5.
33. Skotitisch G, Jacobovitz DM. Calcitonin gene-related peptide coexist with substance P in capsaicin sensitive neurons and sensory ganglia of the rat. Peptides 1985;6(4):747–54.
34. Le Greves P, Nyberg F, Terenius L, Hokfelt T. Calcitonin gene-related peptide is a potent inhibitor of substance P degradation. Eur J Pharmacol 1985;115:309–11.
35. Dubner R, Ruda MA. Activity-dependent neuronal plasticity following tissue injury and inflammation. Trends Neurosci 1992;15:96–103.
36. Hunt SP, Pini A, Evan G. Induction of c-fos-like protein in spinal cord neurons following sensory stimulation. Nature 1987;328:632–34.
37. Fitzgerald M. c-Fos and the changing face of pain. Trends Neurosci 1990;3(11):439–40.
38. Flavell SW, Greenberg ME. Signaling mechanisms linking neuronal activity to gene expression and plasticity of the nervous system. Ann Rev Neurosci 2008;31: 563–90.
39. Lanschulz WH, Johnson PF, McKight SL. The leucine zipper: a hypothetical structure common to a new class of DNA-binding proteins. Science 1988;240:1759–64.
40. Voet D, Voet JG, Pratt CW. Fundamentals of biochemistry, 3rd ed. Hoboken, NJ: John Wiley and Sons, Inc; 2008, pp. 881–2.
41. Sutton MA, Schuman EM. Dendritic protein synthesis, synaptic plasticity, and memory. Cell 2006;127: 49–58.
42. Kennedy MJ, Ehlers MD. Organelles and trafficking machinery for postsynaptic plasticity. Ann Rev Neurosci 2006;29:325–62.
43. Bramham CR, Wells DG. Dendritic mRNA: transport, translation and function. Nat Rev Neurosci 2007;8: 776–89.
44. Portney LG, Watkins MP. Foundations of clinical research: applications to practice, 3rd ed. Upper Saddle River, NJ: Pearson Prentice Hall; 2009, pp. 286, 327.

APPENDIX: EDUCATIONAL RESEARCH QUESTIONNAIRE
Biochemistry Can Be Painful

During the fall 2008 quarter, coursework in Biochemistry I included a description of the role of glutamate as a neurotransmitter. The topic was discussed during an early lecture on amino acids and utilized nociception, molecular details of the pain system, an experimental model (rodent with clamp attached to paw), instructor simulation of the experimental model (ie, paper clamp attached to instructor's fingers), and brief discussion of related topics (ie, neuronal plasticity, central sensitization). This was one of several segments throughout Biochemistry I linking basic biochemistry to the molecular details of the pain system.

Prior to this lecture, were you aware that the amino acid glutamate was a neurotransmitter?
YES _____ NO _____

For the following questions rate your response to the statements provided (Circle the most appropriate description.)

In general, illustrating basic science concepts by utilizing practical and clinical applications is something I appreciate.
Agree Slightly Agree Neutral Slightly Disagree Disagree

This lecture segment describing glutamate as a neurotransmitter in the pain system effectively caught my attention.
Agree Slightly Agree Neutral Slightly Disagree Disagree

The topic of pain was an important factor in capturing my interest regarding glutamate.
Agree Slightly Agree Neutral Slightly Disagree Disagree

The use of a simple experimental model (rodent with paw clamp) was a useful tool to give context to glutamate's role as a neurotransmitter.
Agree Slightly Agree Neutral Slightly Disagree Disagree

Attaching a paper clamp to the instructor's fingers (simulating the experimental model) was useful in making the presentation memorable.
Agree Slightly Agree Neutral Slightly Disagree Disagree