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Hypothesis

Endogenous Opioids Contain Terminal Amino Acids That May Be Transformed to Catecholamines *In Vivo* and Modulate Pain

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Abstract

Feedback loops or compensatory mechanisms are present in a wide range of biological systems and processes. Here, we hypothesize that endogenous opioid peptides, such as endorphins and enkephalins, can be broken down and enzymatically converted to catecholamines (dopamine, norepinephrine, epinephrine) locally. Particularly, this proposed local production of norepinephrine may modulate analgesia through feedback mechanisms. A similar arrangement may occur for corticotropin-releasing factor and adrenocorticotropic hormone (hypothalamic-pituitary-adrenal axis mediation), insulin (blood glucose regulation), and angiotensin II (cardiovascular regulation). Endorphins, enkephalins, and dynorphins have an initial amino acid sequence of Tyr-Gly-Gly-Phe, where tyrosine (and possibly phenylalanine) could be enzymatically clipped from the peptide and converted to catecholamines locally, through the canonical biosynthetic molecular pathway for catecholamines. Spatially and possibly temporally precise conversion of these terminal amino acids to catecholamines may allow them to be produced "on demand" in specific regions of the brain, spinal cord, or periphery. This hypothesis is readily testable by infusing stable isotopically labeled opioids into the brain or periphery of model organisms, and observing through liquid chromatography-mass spectrometry whether the terminal amino acids of these opioids are converted to catecholamines.

Introduction

Compensatory mechanisms or feedback loops, including positive and negative feedback regulation, occur in a broad range of biological systems and processes. For example, a major output molecule of the human hypothalamic-pituitary-adrenal axis, cortisol, is engaged in negative feedback regulation that can reduce its own production.[1] Feedback regulation may also characterize neurophysiological responses to pharmacological agents, including upregulation of D2 dopamine receptors in response to chronic treatment with antipsychotic drugs.[2] Compensatory processes may even correlate with behavioral measures in rodents, where upregulation of neural activity in medial prefrontal cortex may represent a failed response to counteract impaired extinction in a fearful strain of mice.[3] The current publication suggests that various feedback mechanisms may be involved in novel pathways for neurotransmitter biosynthesis, in a range of organisms.

Hypothesis

The locus coeruleus-norepinephrine system plays a vital role in pain modulation, both through its connections within the brain and its descending inputs to the spinal cord.[4] Here, we hypothesize that endogenous opioid peptides, such as endorphins and enkephalins, which have analgesic properties, can be broken down and enzymatically converted to catecholamines (dopamine, norepinephrine, epinephrine) locally. In this way, local production of norepinephrine in particular may provide feedback for keeping analgesia from endogenous opioids in check, or otherwise modulating analgesia. The biosynthetic pathway from tyrosine to catecholamines has indeed been established over 70 years ago,[5–7] but to our knowledge it is not thought to operate in the precise, "on demand" manner proposed here. Consistent with a previous publication on stimulation-produced analgesia,[8] perhaps local brain or spinal cord production of norepinephrine could provide feedback to amplify pain and counteract analgesia produced by endogenous opioids. An alternative hypothesis is that local biosynthesis of catecholamines from endogenous opioids may contribute to analgesia, or perhaps this

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production can either increase or decrease local pain in different circumstances.

Endorphins, enkephalins, and dynorphins all have an initial amino acid sequence of Tyr-Gly-Gly-Phe,[9] where tyrosine could be enzymatically clipped from the peptide and transformed to catecholamines in a local manner. The two glycines after tyrosine in the sequence are the smallest amino acids and may allow tyrosine to be enzymatically clipped since they may not sterically hinder the clipping enzyme. Phenylalanine, a known biosynthetic precursor to tyrosine, in the fourth position may also be excised and converted to catecholamines. Spatially and possibly temporally precise conversion of these terminal amino acids to catecholamines may allow them to be produced "on demand" in specific regions of the brain, spinal cord, or periphery. The related molecules nociceptin and the endomorphins have an amino acid sequence that respectively begins with phenylalanine or tyrosine,[9] which may also facilitate bioconversion to catecholamines. However, Substance P does not possess such a characteristic amino acid sequence.

Corticotropin-releasing factor (CRF) and adrenocorticotropic hormone (ACTH) also have particular amino acids on their ends, such as tyrosine and serine,[10,11] that may be excised and converted to catecholamines *in vivo*. These hormones are involved in the hypothalamic-pituitary-adrenal axis response to psychological stress, which is partially mediated by norepinephrine and epinephrine. A similar principle may apply to insulin, with its A and B sequences, where phenylalanine is the first amino acid in one of these peptide sequences (B). Also, at the other end of the B peptide, there is a Tyr-Phe-Phe sequence four amino acids in. This latter sequence could be enzymatically excised and converted to catecholamines as well. Since insulin decreases the blood glucose level whereas norepinephrine can increase it,[12] local conversion of insulin to norepinephrine could represent a compensatory mechanism to regulate glucose availability. The angiotensin II peptide, which plays a prominent role in cardiovascular regulation, also has phenylalanine at one of its terminals. Since angiotensin II can increase blood pressure,[13] it could amplify catecholaminergic signaling by being transformed to norepinephrine or epinephrine locally. On the other hand, such putative local production of catecholamines from angiotensin II could produce a paradoxical compensatory effect on blood pressure.

Evaluation of the hypothesis

Stable isotope biochemical experiments, which do not involve radioactivity and are accordingly safe to carry out, can be used to test the overarching hypotheses about endogenous opioids described here.[14] The same principles can be applied to examine the amino acids in the other molecules reviewed here, such as angiotensin II and CRF. For example, tyrosine or phenylalanine within the beta endorphin molecule can be engineered to contain carbon-13 or deuterium in one or more of its atoms (for example, synthesized by: MilliporeSigma, CDN Isotopes, Charles River Laboratories, Cambridge Isotope Laboratories, KareBay Biochem), and this "heavy", labeled beta endorphin can then be infused (intracerebroventricularly) into the brain of a mouse or a rat. After a delay of minutes to hours or even up to a day or so, microdialysis samples can be obtained and analyzed using liquid chromatography-mass spectrometry to test for the presence of carbon-13 (or deuterium) labeling within dopamine, norepinephrine, and epinephrine molecules. Control animals can receive unlabeled carbon-12 (or regular hydrogen) beta endorphin, where the carbon-13/carbon-12 ratio for each catecholamine can then be compared between the experimental and control animals using an unpaired two-tailed t-test. Microdialysis may be needed, rather than less sensitive assays, to detect the presumably very low concentrations of labeled catecholamines *in vivo*. Systemic (intraperitoneal, subcutaneous, or intravenous) administration of carbon-13 (or deuterium) labeled beta endorphin could also be carried out, followed by testing blood plasma, urine, or brain samples.

Additional considerations

Several other points should also be considered regarding the overarching hypothesis presented here. First, why would analgesia from endogenous opioids need to be kept in check by putative biotransformation to catecholamines such as norepinephrine? As noted above, local production of norepinephrine may actually increase analgesia rather than counteract it, and in either case, it may serve as an additional means for pain modulation, allowing for more flexible regulation. Additional consequences may result from biotransformation of endogenous opioids to catecholamines, where one possibility is that the endogenous opioid and catecholaminergic systems work more closely together *in vivo* than currently appreciated. Finally, a potentially deleterious outcome of the hypothesis proposed here, may be that dysregulation of the endogenous opioid system could disrupt many other endogenous systems and signaling pathways. While this could be the case, another possibility is that precise temporal and spatial control over the production of catecholamines, as suggested here, would limit disturbances in other molecular systems.

Future directions

There are a number of possibilities for extending the ideas put forth in this publication. First, the series of hypotheses described here need to be directly tested with the straightforward techniques described above. If these hypotheses are experimentally confirmed, a future direction would be to more precisely determine the anatomical locations and cell types within the body where these biochemical processes occur, where one medium is the bloodstream itself, or various circuits within the brain, for example. Of critical importance would be to identify the potentially novel enzymes that carry out these putative reactions, particularly the enzymes that may clip the various peptides described above, allowing for conversion to catecholamines. These enzymes could be druggable targets for disorders of pain in the case of the endogenous opioids, metabolic disorders in the case of insulin, or cardiovascular disorders with regard to angiotensin II.

Conclusions

This publication has put forth the hypothesis that terminal amino acids of endogenous opioid peptides may be enzymatically cleaved and converted to catecholamines *in vivo*, in a spatially and possibly temporally precise manner. This novel form of regulation may allow for "on demand" production of these important signaling molecules that could help regulate analgesia in a fine-grained manner. Similar reasoning may apply to CRF and ACTH (hypothalamic-pituitary-adrenal axis regulation), insulin (blood glucose supply), and angiotensin II (cardiovascular modulation). Basic researchers using animal models, such as rodents, are encouraged to test these hypotheses using stable isotope pharmacology combined with liquid chromatography-mass spectrometry.

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Conflict of interest

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

PJF is the sole author of the manuscript.

References

[1] Gjerstad JK, Lightman SL, Spiga F. Role of glucocorticoid negative feedback in the regulation of HPA axis pulsatility. Stress 2018;21(5):403–416. doi:10.1080/10253890.2018.1470238, PMID:29764284.

[2] Chouinard G, Samaha AN, Chouinard VA, Peretti CS, Kanahara N, Takase M, Iyo M. Antipsychotic-Induced Dopamine Supersensitivity Psychosis: Pharmacology, Criteria, and Therapy. Psychother Psychosom 2017;86(4):189–219. doi:10.1159/000477313, PMID:28647739.

[3] Fitzgerald PJ, Whittle N, Flynn SM, Graybeal C, Pinard CR, Gunduz-Cinar O, Kravitz AV, Singewald N, Holmes A. Prefrontal single-unit firing associated with deficient extinction in mice. Neurobiol Learn Mem 2014;113:69–81. doi:10.1016/j.nlm.2013.11.002, PMID:24231425.

[4] Tavares I, Costa-Pereira JT, Martins I. Monoaminergic and Opioidergic Modulation of Brainstem Circuits: New Insights Into the Clinical Challenges of Pain Treatment? Front Pain Res (Lausanne) 2021;2:696515. doi:10.3389/fpain.2021.696515, PMID:35295506.

[5] Blaschko H. The activity of l(-)-dopa decarboxylase. J Physiol 1942; 101(3):337–49. doi:10.1113/jphysiol.1942.sp003988, PMID:16991567.

[6] Bulbring E. The methylation of noradrenaline by minced suprarenal tissue. Br J Pharmacol Chemother 1949;4(3):234–244. doi:10.1111/j.1476-5381.1949.tb00542.x, PMID:18141084.

[7] Holtz P. Dopadecarboxylase. Naturwissenschaften 1939;27:724–725. doi:10.1007/BF01494245.

[8] Akil H, Liebeskind JC. Monoaminergic mechanisms of stimulation-produced analgesia. Brain Res 1975;94(2):279–296. doi:10.1016/0006-8993(75)90062-1, PMID:125141.

[9] Li Y, Lefever MR, Muthu D, Bidlack JM, Bilsky EJ, Polt R. Opioid glycopeptide analgesics derived from endogenous enkephalins and endorphins. Future Med Chem 2012;4(2):205–226. doi:10.4155/fmc.11.195, PMID:22300099.

[10] Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science 1981;213(4514):1394–1397. doi:10.1126/science.6267699, PMID:6267699.

[11] Fitzgerald PJ. Neurodining: Common dietary factors may be substrates in novel biosynthetic pathways for monoaminergic neurotransmitters. Med Hypotheses 2020;138:109618. doi:10.1016/j.mehy.2020.109618, PMID:32070787.

[12] Thorens B. Neuronal regulation of glucagon secretion and gluconeogenesis. J Diabetes Investig 2022;13(4):599–607. doi:10.1111/jdi.13745, PMID:34989155.

[13] Wu H, Sun Q, Yuan S, Wang J, Li F, Gao H, *et al*. AT1 Receptors: Their Actions from Hypertension to Cognitive Impairment. Cardiovasc Toxicol 2022;22(4):311–325. doi:10.1007/s12012-022-09730-0, PMID:35211833.

[14] Fitzgerald PJ. Many Drugs of Abuse May Be Acutely Transformed to Dopamine, Norepinephrine and Epinephrine In Vivo. Int J Mol Sci 2021;22(19):10706. doi:10.3390/ijms221910706, PMID:34639047.