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EDITORIAL

Is Assigning a Descriptor with Definitive Functional or Clinical Annotation to Molecules or Signaling Pathways A Hindrance to Advancements in Biomedical Research?

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A common practice in contemporary biomedicine is to assign a descriptor with definitive functional or clinical annotation to a particular molecule or signaling pathway. Thus, terms such as tumor suppressor molecules or survival signaling pathways abound in the literature. Despite its simplicity and convenience, this practice may not be a true reflection of how human bodies function. This is because manifestations of body functions represent the outcomes of orchestrated events that are integrated at multiple levels of systems, organs, tissues, cells, and molecules. When these multilevel integrations are executed in "good" rapport, our body functions are operated in the "physiological" zone. "Pathophysiological" conditions will be instigated when they turn into "bad" relationships, leading to the development of diseases. The "ugly" scenario will emerge on the breakdown of the multilevel integration system, which prompts "pathological" states that head for fatality.

A "good" molecule/signaling pathway under physiological conditions can thus become a "bad" or "ugly" molecule/signaling pathway (or vice versa) under pathophysiological or pathological circumstances. Using the role of nitric oxide (NO) signaling in the control of blood pressure (BP) as an illustrative example, this editorial epitomizes the risk of hindering the advancement of biomedical research by assigning definitive functional or clinical annotation to any molecule or signaling pathway.

Nitric Oxide the Molecule

The gaseous molecule nitric oxide (NO) captured the spotlight of contemporary research in biomedicine when Robert F. Furchgott, Louis J. Ignarro and Ferid Murad were jointly awarded the Nobel Prize in Physiology or Medicine in 1998 for their discoveries concerning "nitric oxide as a signaling molecule in the cardiovascular system". NO is synthesized by NO synthase (NOS), which uses NADPH and oxygen to convert a guanidino nitrogen of L-arginine to yield citrulline as a product, along with NO [1]. Four NOS isoforms have been identified in mammalian cells, namely neuronal NOS (nNOS or NOS I), inducible NOS (iNOS, NOS II), endothelial NOS (eNOS or NOS III), and mitochondrial NOS (mtNOS). It is generally accepted that both NOS I and NOS III are constitutively expressed primarily in nerve and endothelial cells, respectively, and are responsible for basal NO release in a calcium-calmodulin dependent manner; whereas NOS II is an inducible form initially identified in macrophages for the generation of NO in a calcium-independent fashion [2]. The mtNOS is a relatively new member of the NOS family and was identified as an isoform of constitutive NOS I present in the inner mitochondrial membrane [3].

Control of Blood Pressure Engages Multilevel Integration

Maintenance of a stable BP requires integration at the level of systems (neural, hormonal, humoral and

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immune systems); organs (heart, blood vessel, kidney and brain); and cells (endothelial cells, smooth muscle cells, neurons, immune cells and perivascular adipocytes). An array of molecules, including at least NO, angiotensin II, endothelin, vasopressin and superoxide of the reactive oxygen species (ROS), are known to be engaged in those integrative processes.

Nitric Oxide as a "Good" Molecule

The overall evidence that emerged from work published during the last three decades suggests that NO released under physiological conditions is considered to be a "good" molecule. It is in essence engaged in both tonic and reflex control of BP homeostasis, as well as cardiovascular adaptations under conditions such as physical exercise and emotional stress. The most recognizable action of NO, which instigates clinical translation of the molecule, is that on generation from NOS III in the endothelial cells, NO diffuses into the underlying vascular smooth muscle cells where it causes elevation of cyclic guanosine monophosphate (cGMP) through the activation of cytosolic guanylate cyclase [4]. Activation of calcium-dependent potassium channels by cGMP-dependent protein kinases and phosphorylation of myosin in vascular smooth muscle cells are the two salient events responsible for the well-known NO-induced vasorelaxation [4]. These ground-breaking findings also led to the realization that under physiological conditions, continuous basal release of NOS III-derived NO maintained by neurotransmitters such as acetylcholine and humoral factors such as bradykinin is responsible for the vasodilator tone of vessels [5].

Endothelial NO is also vasoprotective. NO released towards the vascular lumen is a potent inhibitor of platelet aggregation and adhesion to the vascular wall [6]. It also prevents vascular smooth muscle proliferation by inhibiting the release of platelet-derived growth factors.

Soon after the characterization of the basic biological actions of NO in the vasculature, evidence of NO as a physiological mediator in other tissues and organs involved in the maintenance of BP homeostasis emerged. NO is now established to be a key neuromodulator within the central nervous system, particularly in autonomic regions involved in neural and neurohormonal control of circulation, including nucleus tractus solitarii (NTS), rostral and caudal ventrolateral medulla (RVLM and CVLM) and hypothalamic paraventricular nucleus (PVN). For example, NOS III-derived NO in the PVN exerts tonic inhibition on sympatho-excitatory outflow [7], and NO derived from NOS III in the NTS tonically inhibits baroreflex feedback control of BP [8]. Moreover, simultaneous sympatho-excitatory and sympatho-inhibitory effects of NOS III-derived NO within the RVLM and CVLM constitute a part of physiological adaptations in the central control of the circulation during muscle contraction and static exercise [9].

Both NOS I and NOS II within the central nervous system are also engaged in the neural control of BP under physiological conditions. Contemporary literature supports an excitatory role for NOS I-generated NO at the PVN in regulating sympathetic vasomotor outflow [10] and modulating baroreflex sensitivity in the NTS [11]. NOS II, as the name denotes, is originally thought to require activation in macrophages, astrocytes, and microglia by immunological or inflammatory stimuli. There is now evidence that NOS II is also expressed constitutively in neurons and microglia. In the RVLM, NO derived from NOS I causes sympatho-excitation via activation of glutamatergic neurotransmission, whereas NOS II-derived NO promotes sympatho-inhibition via stimulation of GABAergic neurotransmission [10,12]. Moreover, the activity of NOS I is more prevalent than NOS II under physiological conditions. By eliciting sympatho-inhibition, NO produced by the tonically active NOS II becomes the "good" molecule because it is crucial to the maintenance of normal BP by counter-balancing the sympatho-excitatory actions of NOS I-derived NO in the RVLM [12]. The RVLM neurons are also engaged in baroreflex regulation of sympathetic nerve activity. In this regard, NOS I in the RVLM contributes to the processing of the cardiac sympatho-excitatory reflexes [13] and facilitate sympathetic baroreflex transmission [14]. In addition, NOS II within the RVLM plays an active role in modulating sympatho-excitation in exercise pressor reflex [15].

Given its indispensable role in the maintenance of BP homeostasis, a reduction in tissue NO bioavailability would in theory lead to "bad"

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consequences of cardiovascular functions. Indeed, one interesting and definitive conclusion from more than 120,000 scientific publications on NO during the last thirty years is that individuals with clinically diagnosed atherosclerosis, diabetes, or hypertension often show impaired NO signaling pathways. For example, hypercholesterolemia and atherosclerosis are associated with impairment of endothelium-mediated vasodilatation in the vasculature, mainly due to increased circulating levels of the endogenous NOS inhibitor asymmetric dimethyl-L-arginine [16]. A blunted vasodilatation in response to acetylcholine has also been documented in both animal models of and patients with hypertension. A variable number of tandem repeats in intron 4 [17] and a missense variant, Glu298Asp, in exon 7 [18] of the NOS III gene were found to be significantly associated with human essential hypertension. Later work in animal studies demonstrated that endothelial NO deficiency may result in an abnormal vascular phenotype and instigate pathological changes in the vessel wall associated with hypertension [19] and atherosclerosis [20].

Nitric Oxide as a "Bad" Molecule

A literature survey suggests that NO in the brain exhibits both neuroprotective and neurotoxic effects on central circulatory control that is dependent on the pathophysiological stages. For example, during the early stages of cerebral ischemia, a surge in NO release generated by NOS III seems to protect neurons from death by inducing vasodilatation and inhibiting microvascular aggregation [21]. However, the overproduction of NO by NOS I or NOS II in the later stages of stroke contributes to apoptosis and subsequent neuronal death [5]. Activation of NOS II precipitates a decrease in BP following cytokine release induced by the endotoxin, *E. coli* lipopolysaccharide [22].

Further investigations revealed that the various biological actions of NO are likely dependent on the gas production kinetics of the different NOS isoforms. The constitutive NOS (i.e., NOS I and NOS III) produces pulsatile release of very small (in nM range) amounts of NO; whereas NOS II is responsible for the generation of a larger amount of NO (in μM range) release over longer periods [23]. As such, NO that plays a physiological role in BP regulation when it is produced by the constitutive NOS could become a pathophysiological entity when generated by NOS II. Although the exact amount of NO produced *in situ* by each of the NOS isoforms under physiological or pathophysiological state cannot be accurately quantified, evidence shows that when activated, one NOS II molecule can generate several hundred to several thousand times more NO than one NOS I molecule [24]. This excessive amount of NO released in tissues could well be responsible for the "bad" face of the molecule in the control of BP.

Nitric Oxide as an "Ugly" Molecule

The "redox nature" and cytotoxic actions of NO did not attract much attention from the physiology community when Blough and Zafiriou [25] made the original demonstration that NO can react with superoxide (a member of ROS family) in aqueous solution to yield peroxynitrite anion, a potent biological oxidant and reactive nitrogen species. One reason is that oxidative stress and nitrosative stress are frequently depicted as the common culprits for cellular damage. In addition, the expression "ROS/RNS" has often appeared in the literature as if they represent a singular moiety, usually as the surrogate for ROS [26]. In fact, the reaction between NO and superoxide was initially taken by the physiological community as a way to "regulate" the biological half-life of NO; a reaction perceived as an "oxidative inactivation" of NO for its degradation to yield unreactive products, mainly nitrate and nitrite. It was the much later discovery of peroxynitrite as a biologically relevant cytotoxic intermediate [27] that spurred research on the "ugly" side of NO.

In the context of BP control, the markers of peroxynitrite generation have not only been documented in animal models of shock, but also in human specimens obtained from patients suffering from circulatory shock [28,29]. There is a significant correlation between the degree of nitrotyrosine formation and the severity of the clinical condition in human sepsis. We now know that peroxynitrite, primarily generated via the reaction of the NOS II-induced NO and superoxide, plays a significant pathogenetic role at least in vascular hyporeactivity,

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capillary extravasation, tissue edema, myocardial hypocontractility, pulmonary and renal injury associated with circulatory shock [30]. Under a pathological condition exemplified by experimental brain stem death, the overproduction of NO generated by massive activation of NOS II in the RVLM, coupled with the impending augmentation of superoxide, elicits presynaptic inhibition on glutamate release through the formation of peroxynitrite, leading to prolonged sympatho-inhibition that results in severe hypotension [26,31].

Concluding Remarks

For the sake of simplicity and convenience, it is not uncommon for biomedical scientists to label NO as a "good", "bad" or "ugly" molecule. As pointed out in this editorial, this is conceptually contentious. Whether NO acts as a "good" or "bad" signaling molecule or an "ugly" cytotoxic agent depends largely on its concentration and the redox environment at the site where its actions take place, and are contingent on its engagements in a physiological, pathophysiological or pathological process. The body is economical in that the same molecular pathways are used in different body systems. It follows that assigning a descriptor with definitive functional or clinical annotation to any molecule or signaling pathway is not only inadvisable but may impede the progress of biomedical research because it may discourage investigators from pursuing "unexpected" results that do not conform to the "annotated" functional or clinical engagement of the molecule or signaling pathway in question.

REFERENCES

1. Knowles RG, Moncada S. Nitric oxide synthases in mammals. Biochem J. 1994;298 (Pt 2):249-258.
2. Förstermann U, Kleinert H, Gath I, Schwarz P, Closs EI, Dun NJ. Expression and expressional control of nitric oxide synthases in various cell types. Adv Pharmacol. 1995;34:171-86.
3. Elfering SL, Sarkela TM, Giulivi C. Biochemistry of mitochondrial nitric-oxide synthase. J Biol Chem. 2002;277: 38079-38086.
4. Ignarro LJ. Endothelium-derived nitric oxide: actions and properties. FASEB J. 1989;3:31-36.
5. Bredt DS. Endogenous nitric oxide synthesis: biological functions and pathophysiology. Free Radic Res. 1999;31:577-596.
6. Radomski MW, Palmer RM, Moncada S. Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. Lancet. 1987;2:1057-1058.
7. Biancardi VD, Son SJ, Sonner PM, Zheng H, Patel KP, Stern JE. Contribution of central nervous system endothelial nitric oxide synthase to neurohumoral activation in heart failure rats. Hypertension. 2011;58:454-463.
8. Waki H, Kasparov S, Wong LF, Murphy D, Shimizu T, Paton JF. Chronic inhibition of endothelial nitric oxide synthase activity in nucleus tractus solitarii enhances baroreceptor reflex in conscious rats. J Physiol (Lond). 2003;546:1-42.
9. Ishide T, Preuss CV, Maher TJ, Ally A. Neurochemistry within ventrolateral medulla and cardiovascular effects during static exercise following eNOS antagonism. Neurosci Res. 2005;52:21-30.
10. Chan SHH, Chan JYH. Brain stem NOS and ROS in neural mechanisms of hypertension. Antioxid Rex Signal. 2014;20:146-163.
11. Lin LH, Nitschke Dragon D, Jin J, Tian X, Chu Y, Sigmund C, Talman WT. Decreased expression of neuronal nitric oxide synthase in the nucleus tractus solitarii inhibits sympathetically mediated baroreflex responses in rat. J Physiol. 2012;590:3545-3559.
12. Chan SHH, Wang LL, Chan JYH. Differential engagements of glutamate and GABA receptors in cardiovascular actions of endogenous nNOS or iNOS at rostral ventrolateral medulla of rats. Br J Pharmacol. 2003;138:584-593.
13. Guo ZL, Tjen-A-Looi SC, Fu LW, Longhurst JC. Nitric oxide in rostral ventrolateral medulla regulates cardiac sympathetic reflexes: role of synthase isoforms. Am J Physiol Heart Circ Physiol. 2009;297:H1478-H1486.

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https://doi.org/10.24191/jchs.v8i2.23680

14. Mayorov DN. Selective sensitization by nitric oxide of sympathetic baroreflex in rostral ventrolateral medulla of conscious rabbits. Hypertension. 2005;45:901-906.
15. Ally A, Phattanarudee S, Kabadi S, Patel M, Maher TJ. Cardiovascular responses and neurotransmitter changes during static muscle contraction following blockade of inducible nitric oxide synthase (iNOS) within the ventrolateral medulla. Brain Res. 2006;1090:123-133
16. Miyazaki H, Matsuoka H, Cooke JP. Endogenous nitric oxide synthase inhibitor: a novel marker of atherosclerosis. Circulation. 1999;99:1141-1146.
17. Uwabo J, soma M, Nakayama t, Kanmatsuse K. Association of a variable number of tandem repeats in the endothelial constitutive nitric oxide synthase gene with essential hypertension in Japan. Am J Hypertens. 1998;11:125-128.
18. Miyamoto Y, Saito Y, Kajiyama N, Yoshimura M, Shimasaki Y, Nakayama M, Kamitani S, Harada M, Ishikawa M, Kuwahara K, Ogawa E, Hamanaka I, Takahashi N, Kaneshige T, Teraoka H, Akamizu T, Azuma N, Yoshimasa Y, Yoshimasa T, Itoh H, Masuda I, Yasue H, Nakao K. Endothelial nitric oxide synthase gene is positively associated with essential hypertension. Hypertension. 1998;32:3-8.
19. Paravicini TM, Touyz RM. Redox signaling in hypertension. Cardiovasc Res. 2006;71:247-258.
20. Kawashima S, Yokoyama M. Dysfunction of endothelial nitric oxide synthase and atherosclerosis. Arterioscler Thromb Vasc Biol. 2004;24:998-1005.
21. Zhou L, Zhu DY. Neuronal nitric oxide synthase: structure, subcellular localization, regulation, and clinical implications. Nitric Oxide. 2009;20:223-230.
22. Chan JYH, Chang AYW, Wang LL, Ou CC, Chan SHH. Protein kinase C-dependent mitochondrial translocation of proapoptotic protein Bax on activation of inducible nitric-oxide synthase in rostral ventrolateral medulla mediates cardiovascular depression during experimental endotoxemia. Mol Pharmacol. 2007;71:1129-1139.
23. Granger DL, Hibbs JB Jr, Perfect JR, Durack DT. Metabolic fate of L-arginine in relation to microbiostatic capability of murine macrophages. J Clin Invest. 1990;85:264-273.
24. Pannu R, Singh I. Pharmacological strategies for the regulation of inducible nitric oxide synthase: neurodegenerative versus neuroprotective mechanisms, Neurochem. Int. 2006;49:170-182.
25. Blough NV, Zafiriou OC. Reaction of superoxide with nitric oxide to form peroxonitrite in alkaline aqueous solution. Inorg Chem. 1985;24:3502-3504.
26. Chan JYH, Chan SHH. Differential impacts of brain stem oxidative stress and nitrosative stress on sympathetic vasomotor tone. Pharmacol Therapeut. 2019;201:120-136.
27. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: Implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci. USA. 1990;87:1620-1624.
28. Levy B, Collin S, Sennoun N, Ducrocq N, Kimmoun A, Asfar P, Perez P, Meziani F. Vascular hyporesponsiveness to vasopressors in septic shock: from bench to bedside. Intensive Care Med. 2010;36:2019-2029.
29. Szabó C, Módis K. Pathophysiological roles of peroxynitrite in circulatory shock. Shock. 2010;34(Suppl 1):4-14.
30. Radi R. Oxygen radical, nitric oxide, and peroxynitrite: Redox pathway in molecular medicine. Proc Natl Acad Med USA. 2018;115:5839-5848.
31. Chan SHH, Chan JYH. Brain stem oxidative stress and its associated signaling in the regulation of sympathetic vasomotor tone. J Appl Physiol. 2012;113:1921-1928.

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