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Epinephrine Biosynthesis: Hormonal and Neural Control During Stress

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SUMMARY

1. Stress contributes to the pathophysiology of many diseases, including psychiatric disorders, immune dysfunction, nicotine addiction and cardiovascular illness. Epinephrine and the glucocorticoids, cortisol and corticosterone, are major stress hormones.

2. Release of epinephrine from the adrenal medulla and glucocorticoids from the adrenal cortex initiate the biological responses permitting the organism to cope with adverse psychological, physiological and environmental stressors. Following its massive release during stress, epinephrine must be restored to replenish cellular pools and sustain release to maintain the heightened awareness and sequelae of responses to re-establish homeostasis and ensure survival.

3. Epinephrine is regulated in part through its biosynthesis catalyzed by the final enzyme in the catecholamine pathway, phenylethanolamine *N*-methyltransferase (E.C. 2.1.1.28, PNMT). PNMT expression, in turn, is controlled through hormonal and neural stimuli, which exert their effects on gene transcription through protein stability.

4. The pioneering work of Julius Axelrod forged the path to our present understanding of how the stress hormone and neurotransmitter epinephrine, is regulated, in particular via its biosynthesis by PNMT.

KEY WORDS: Epinephrine; PNMT; stress; hormonal regulation; neural regulation.

INTRODUCTION

My lineage places me as a "scientific grandchild" of Julie's, as my mentor, the late Roland Ciaranello, had the privilege of working with Julie as a medical student and subsequently as postdoctoral fellow after completing his MD at Stanford University School of Medicine. I recall my first interaction with Julie at an International Catecholamine Meeting at Asilomar, CA. How should I treat a Nobel Laureate? What could I talk about and not embarrass both him and myself? Julie quickly allayed those concerns, and I enjoyed a goodly portion of an evening at the Monterey Aquarium in conversation ranging from science to life.

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Perhaps one of the most important things that Julie may have passed on to all of his extended scientific family is the notion of "inquisition and pursuit," a passion for not merely accepting our findings at face value but actively extending far beyond. Through his inquisition and pursuit of the catecholamine neurotransmitter and neurohormone epinephrine and its biosynthetic enzyme, phenylethanolamine *N*-methyltransferase (E.C. 2.1.1.28, PNMT), a remarkable foundation of knowledge was established, and the latter has formed the basis of several decades of investigation in my laboratory. Thank you, Julie, for providing the encouragement to aspire and paving the pathway for so doing.

Julie and coworkers contributed much to our understanding of how epinephrine and PNMT are regulated via neural stimuli, hormonal stimuli and stress. This review spans from those pioneering studies through present knowledge of mechanisms by which stress-activated hormonal and neural pathways contribute to the genetic control of epinephrine biosynthesis via PNMT. While hormones and neurotransmitters are important regulators of PNMT, they exert their effects by activating the hypothalamic–pituitary–adrenal (HPA) axis or stress axis and the splanchnic nerve innervating the adrenal medulla.

Hormonal Regulation of Epinephrine Via Its Biosynthesis

Epinephrine is generated from norepinephrine by N-methylation, a process catalyzed by PNMT (Axelrod, 1962), utilizing as methyl donor, S-adenosylmethionine (AdoMet, Fig. 1). Early investigations by Wurtman and Axelrod (1971) showed that PNMT is predominantly glucocorticoid-regulated, orchestrated via the pituitary gland and adrenal cortex (Wurtman and Axelrod, 1965). In many species the adrenal medulla, the major site of peripheral epinephrine production, is contiguous with the adrenal cortex, the site of corticosteroid production. Hence, the latter is perhaps not surprising. Pituitary ablation, which eliminates the source of ACTH and thereby prevents corticosteroid production, depletes PNMT expression to approximately 20% of basal values. Expression can be fully restored to normal levels, but not beyond, through corticosteroid replacement. These first studies provided important information about the downstream effects of glucocorticoids on PNMT but little insight as to the mechanism(s) by which they achieved these effects.

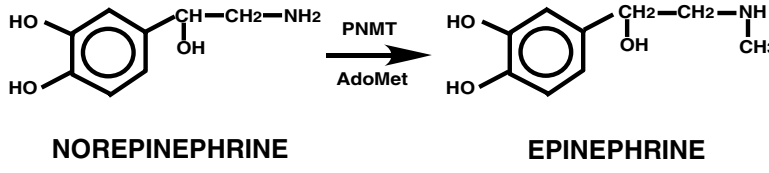

Fig. 1. Epinephrine biosynthesis. Phenylethanolamine *N*-methyltransferase (PNMT), the final enzyme in the catecholamine biosynthetic pathway, produces epinephrine from norepinephrine using as co-substrate and methyl donor *S*-adenosylmethionine.

Subsequently, we showed that one mechanism by which glucocorticoids controlled PNMT was post-translationally by indirectly preventing the degradation of the PNMT enzyme (Berenbeim *et al.*, 1979; Wong *et al.*, 1982). *In vitro*, AdoMet protected PNMT against thermal degradation. In rats, corticosteroids controlled levels of the AdoMet metabolic enzymes, methionine adenosyltransferase and *S*-adenosylhomocysteine hydrolase. By sustaining expression of the latter enzymes, sufficient AdoMet was available for catalysis and binding of this co-substrate stabilized PNMT by masking proteolytically vulnerable regions of the protein.

Another way in which glucocorticoids control PNMT is by regulating the expression of PNMT mRNA (Evinger *et al.*, 1992; Wong *et al.*, 1992; Wong *et al.*, 1995). Hypophysectomy has been reported to deplete rat adrenal PNMT mRNA while glucocorticoid administration restores message levels towards normal values (Evinger *et al.*, 1992). Although we have not observed a similar decline in PNMT mRNA following pituitary ablation (Wong *et al.*, 1995), corticosteroid administration can elevate PNMT mRNA well beyond normal expression (>20-fold) (Wong *et al.*, 1992). However, commensurate changes in PNMT enzymatic activity do not occur, indicating that post-transcriptional regulatory controls impose additional restrictions on enzyme expression, and it may well be that the latter includes changes in PNMT protein stability.

Furthermore, differences in PNMT mRNA appear orchestrated through gene expression. When the rat PNMT gene was originally cloned (Ross *et al.*, 1990), a glucocorticoid response element (GRE) was identified at −533 bp in the proximal upstream promoter sequences. Puzzling, however, was the inconsistency with which glucocorticoid stimulation of PNMT promoter-reporter gene constructs could be evoked in transfected cells. Subsequently, we identified two GREs at −759 and −773 bp upstream of the transcription initiation site in the PNMT promoter, which appear to be the critical GREs associated with corticosteroid regulation of the PNMT gene (Tai *et al.*, 2002). A dose-dependent rise in PNMT promoter-driven reporter gene activity is apparent in response to the type II glucocorticoid receptor (GR) agonist, dexamethasone. Activation can be blocked by the type II GR antagonist, RU38486, thereby demonstrating that classical GRs mediate corticosteroid-stimulated transcriptional activity. When the GREs in the rat PNMT promoter are mutated, either independently or in combination, a loss of glucocorticoid activation occurs, consistent with their altering PNMT gene transcription. We have further shown that glucocorticoid/GR-driven transcription can be synergistically enhanced through interaction of activated, promoter-bound GRs with other PNMT promoter-bound transcriptional inducers. In particular, glucocorticoid-activated GRs bound to the −759 and −773 bp GREs can interact with the immediate early gene transcription factor Egr-1 and/or the developmental factor AP-2, a factor critical for differentiation of neural crest derived tissue, e.g., adrenal medulla (Wong *et al.*, 1998b; Tai *et al.*, 2002). The originally identified −533 bp GRE also appears to participate in synergistic induction of the PNMT gene when activated GRs are bound, but only in association with AP-2.

Recently, we have demonstrated that transcriptional stimulation of PNMT promoter-driven gene transcription by glucocorticoids may, at least in part, involve cAMP cell signaling, either in conjunction with or independent of protein

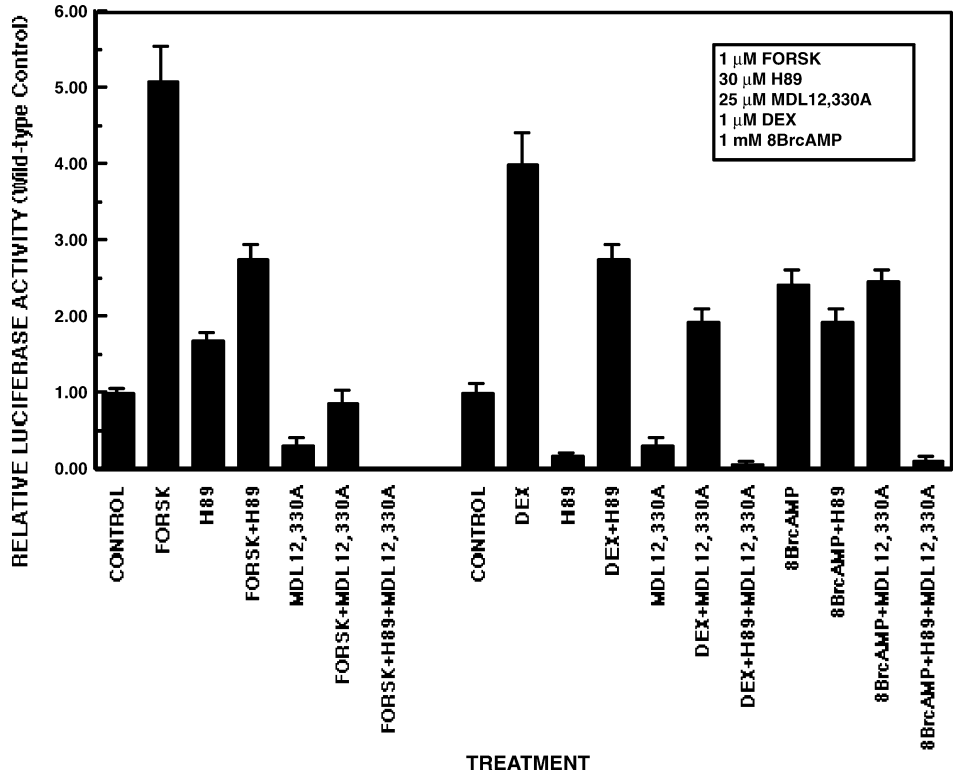

Fig. 2. cAMP and protein kinase A signaling in glucocorticoid-stimulated PNMT promoter activity. PC12 cells transfected with the rat PNMT promoter-luciferase reporter gene construct harboring −893 bp of rat PNMT upstream promoter sequence linked to the firefly luciferase reporter gene (pGL3RP893) were cultured in DMEM containing 5% each of charcoal depleted bovine calf serum and equine serum and 50 μg/ml gentamycin sulfate at 37°C in an atmosphere of 5% CO_2–95% O_2. Cells were pretreated with inhibitors at the concentrations depicted, followed by treatment with 1 μM dexamethsone, and harvested after 24 h for measurement of luciferase activity by luminescence assay. Six replicates were run for each drug treatment. Saline-treated or inhibitor-treated transfected cells served as controls.

kinase A (PKA). The PKA inhibitor H-89 reduces dexamethasone-mediated PNMT promoter driven-luciferase reporter gene expression in PC12 cells 30% while the adenylyl cyclase inhibitor MDL12,330A produces a 50% reduction (Fig. 2, unpublished findings). In addition, if the transfected cells are exposed to dexamethasone, followed by the protein kinase C and Egr-1 activator phorbol 12-myristate 13-acetate (PMA) 20 h later, dexamethasone elicits a decline in PMA-stimulated, Egr-1-mediated PNMT promoter driven transcription (Wong *et al.*, 1996). The latter suggests that PKC signaling may also be associated with glucocorticoid-regulated PNMT transcriptional activity, acting in a reciprocal, modulatory fashion.

Thus, hormones seem to regulate PNMT, and thereby epinephrine, in at least two ways, transcriptionally by controlling the expression of the PNMT gene and post-translationally by regulating the stability of PNMT protein.

Neural Regulation of Epinephrine Via Its Biosynthesis

Axelrod and colleagues in pioneering studies demonstrated that epinephrine synthesis in the adrenal gland is dually controlled by splanchnic innervation. With repeated stimulation, the amount of epinephrine released and residing in adrenal medullary pools exceeds that existing under resting conditions, so that increased neurotransmitter biosynthesis must be occurring (Axelrod and Reisine, 1984). If the splanchnic nerve is reflexively activated by administration of reserpine, a catecholamine re-uptake inhibitor, PNMT activity rises (Thoenen *et al.*, 1970). Elevation can be prevented by splanchnic transsection, indicating that a substance released by pre-ganglionic, sympathetic nerves must underlie the changes in PNMT and epinephrine. These findings set the groundwork for years of investigation examining the role of acetylcholine and cholinergic innervation in regulating PNMT and epinephrine expression. Initial investigations in the adrenal gland demonstrated that PNMT enzyme activity could be induced by acetylcholine through activation of muscarinic and nicotinic receptors and prevented by blockade of protein synthesis (Stachowiak *et al.*, 1990; Wong *et al.*, 1993; Evinger *et al.*, 1994).

Subsequently, it was demonstrated that induction of PNMT enzyme was preceded by a rise in its mRNA (Stachowiak *et al.*, 1990; Wong *et al.*, 1993; Evinger *et al.*, 1994; Morita *et al.*, 1996), and further, that stimulation of message was prevented by the RNA synthesis inhibitor actinomycin D (Wong, unpublished data) and protein by the protein synthesis inhibitor cycloheximide (Morita and Wong, 1996). Thus, acetylcholine apparently functions to activate the PNMT gene. *In vivo* in rats, reserpine produces a rapid rise in adrenal PNMT mRNA (Wong *et al.*, 1993). A single injection elicits maximum induction at 6 h post-drug administration, which is sustained through 12 h. A rise in PNMT mRNA also occurs with repeated reserpine treatment but significant elevation requires at least three successive days of administration. While changes in mRNA are accompanied by increased PNMT protein and enzyme activity, alterations in protein and enzyme are lower in magnitude, indicating that post-transcriptional controls again limit the amount of functional PNMT enzyme.

Subsequently, we demonstrated that the transcription factor Egr-1 contributes to cholinergic induction of PNMT mRNA (Morita *et al.*, 1996; Morita and Wong, 1996). Nicotine, muscarine and carbachol rapidly stimulate Egr-1 mRNA, with PNMT transcriptional activation ensuing. *In vitro*, if the Egr-1 binding element in the PNMT promoter is inactivated by site-directed mutation, transcriptional induction does not occur.

Both PKA and protein kinase C (PKC) signaling appear to mediate neural induction of the PNMT gene (Stachowiak *et al.*, 1990; Evinger *et al.*, 1994; Hwang *et al.*, 1994, 1997). In a PC12 cell line where nicotinic stimulation of PNMT promoter-driven gene expression cannot be evoked, PKA does not contribute to cholinergic responses whereas when both nicotinic and muscarinic cholinergic activation is intact, both pathways participate in cholinergic stimulation of the PNMT promoter (Wong *et al.*, 1998a, 2002a), with Egr-1 mediating PNMT responses. Moreover, PKC effects may depend on prior activation of the PKA pathway (Tai and Wong, 2002, 2003). PMA will not stimulate the PNMT promoter unless PC12 cells transfected

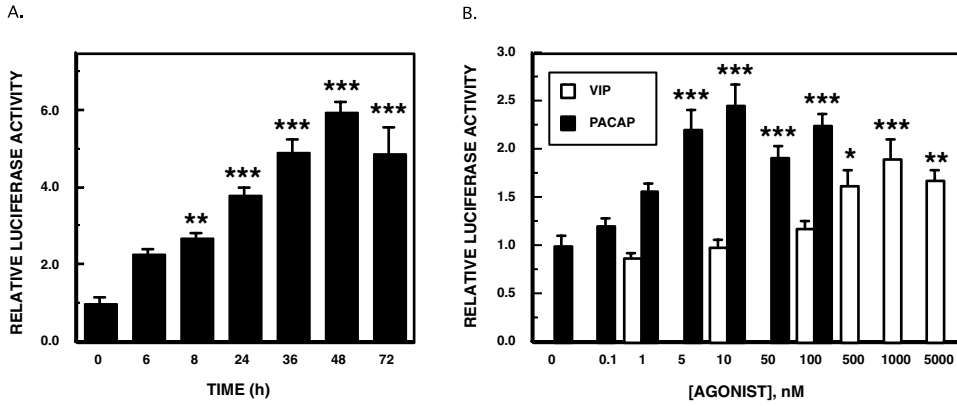

Fig. 3. PACAP-elicited time- and dose-dependent changes in PNMT promoter activity. PC12 cells transfected with pGL3RP893 were maintained as described in Fig. 2. A. Cells were treated with 80 nM PACAP for times through 72 h and luciferase activity measured by luminescence assay. B. Cells were treated with either PACAP or VIP at concentrations from 0 to 5 μM for 24 h and luciferase activity determined. Six replicates were run per treatment group. $^{*}p \leq 0.05$; $^{**}p \leq 0.01$ and $^{***}p \leq 0.001$.

with PNMT promoter-luciferase reporter gene constructs are pre-treated or simultaneously treated with the PKA activator forskolin, with stimulation by the combination fully prevented by the adenylyl cyclase inhibitor MDL12,330A and the PKA inhibitor H-89. Forskolin and PMA together increase Egr-1 protein and its phosphorylation, facilitating Egr-1 binding to the PNMT promoter and thereby transcriptional activation. PKA and PKC pathway activation also increases phosphorylation of existing Sp1 protein, and the latter may contribute to cholinergic induction of PNMT promoter-driven transcriptional activity as well.

Upon stimulation, the splanchnic nerve has been reported to release a variety of neurotransmitters (Coupland and Tomlinson, 1989). However, other than acetylcholine, only pituitary adenylate cyclase activating polypeptide (PACAP) has thus far met the rigorous anatomical and functional criteria qualifying it as a transmitter at the splanchnic-adrenal medullary synapse. This neurotransmitter/neurotrophin has also been shown to induce PNMT (Tonshoff *et al.*, 1997; Choi *et al.*, 1999; Wong *et al.*, 2002a). Its effects are mediated via PKA and PKC signaling as well, with PKA activating and PKC inhibiting PNMT promoter-driven gene transcription (Choi *et al.*, 1999). PACAPergic activation of PNMT is more marked than cholinergic activation and does not seem to desensitize as occurs with the former (Fig. 3(3A), unpublished findings). PACAP stimulates the PNMT promoter in the nM range whereas vasoactive intestinal peptide (VIP), which also activates PACAP receptors, is effective in the μM range, demonstrating that PACAP functions by interacting with high affinity PACAP type I receptors, consistent with their predominant distribution on chromaffin cell membranes and reported association with cAMP/PKA signaling (Fig. 3(3B) and (Wong *et al.*, 2002a; Wong and Tai, 2002)). Downstream of cAMP/PKA signaling, p38 MEK, ERK1/2 MAPK and calcineurin propagate incoming PACAPergic stimulation since inhibitors of these pathways, SB203580, UO126 and ascomycin, attenuate

PACAP induction of the PNMT promoter and their effect in combination with H-89 is no greater than that of H-89 alone. Ca^{2+} appears to be another important second messenger mediating PACAP activation of the PNMT gene promoter. As the L-channel inhibitors D600 and nifedipine and the Ca^{2+}-ATPase pump inhibitor thapsigargin reduce PNMT promoter-driven luciferase expression in PC12 cells, PACAP apparently increases cytosolic Ca^{2+} levels via Ca^{2+} influx through Ca^{2+} L-type voltage sensitive Ca^{2+} channels and release from the endoplasmic reticulum by the Ca^{2+}-ATPase pump. Activation of these pathways, in turn, seems to alter Egr-1 protein and Egr-1 and Sp1 phosphorylation to increase their potential for interaction with cognate binding sites in the promoter and thereby, their ability to activate gene expression (Wong *et al.*, 2002a)

Thus, in contrast to hormonal stimuli, which control PNMT expression via both transcriptional and post-transcriptional regulatory mechanisms, neural stimuli seem to directly activate the PNMT gene and epinephrine biosynthesis only via transcriptional control.

Stress and Epinephrine Biosynthesis

Axelrod and Reisine (1984) described the physiological responses to physical or psychological stress as the release of "adrenocorticotropin (ACTH) from the anterior pituitary, glucocorticoids from the adrenal cortex, epinephrine from the adrenal medulla, and norepinephrine from sympathetic nerves" (Axelrod and Reisine, 1984). Together, these hormones enable organisms to biologically adapt to stressors, ranging from mild to severe, thereby ensuring the organism's survival. In cases of prolonged stress, release of epinephrine and norepinephrine evokes compensatory elevation of the catecholamine biosynthetic enzymes, tyrosine hydroxylase (TH), dopamine β-hydroxylase (DBH) and PNMT, to replenish depleted neurotransmitter/neurohormone stores, and the magnitude and temporal pattern of change in hormonal and neural activity elicited by a particular stress may differentially affect these enzymes. Variation in plasma catecholamine levels evoked by a variety of stressors points to this possibility (Kvetnansky *et al.*, 1984). Results from experimental paradigms combining stress with hypophysectomy, preganglionic denervation of sympathetic nerves or the adrenal medulla and treatment with hormones or neural agents further suggest that induction of TH may be primarily mediated by neural activity, DBH by a combination of neural and hormonal activity and PNMT primarily by hormonal activity (Axelrod, 1962). In the case of PNMT, induction is definitely stressor dependent with variation from tissue to tissue in time course and magnitude (Sabban and Kvetnansky, 2001)

Immobilization (IMMO) stress has been widely utilized in recent years to examine the effects of stress on the catecholamines since IMMO elicits marked changes in these neurotransmitters and their biosynthetic enzymes. Gene transcription seems to be the primary underlying mechanism for short and prolonged or acute and repeated IMMO stress. When rodents are subjected to a single IMMO of 30 or 120 min duration, PNMT is elevated maximally at 6–8 h post-initiation of stress (Viskupic *et al.*, 1994; Wong *et al.*, 2002b, 2004). A similar induction is apparent with

repeated stress for the same lengths of time (Wong *et al.*, 2004). Serum corticosterone levels are still elevated with daily repeated stress through 7 days indicating that the animals have not desensitized to repeated, short or prolonged IMMO. However, while dopamine and its metabolite dihydroxyphenylacetic acid (DOPAC) are elevated, neither norepinephrine nor epinephrine markedly changes. Thus, stimulation of PNMT must provide sufficient enzyme for neurotransmitter synthesis to sustain neurotransmitter pools and neurotransmitter release but not beyond normal levels.

Several signaling pathways have been associated with IMMO stress induction of PNMT mRNA, including the cAMP-PKA, PKC and Ca^{2+} signaling cascades (Sabban and Kvetnansky, 2001). We have further shown that changes in PNMT gene transcription arise via downstream changes in the PNMT transcriptional activator Egr-1, for which increases in mRNA, protein levels and phosphorylation are apparent (Wong *et al.*, 2004). Preliminary findings also indicate that IMMO stress stimulates Sp1 protein expression and phosphorylation as well although two other PNMT transcriptional activators, the GR and AP-2, remain unchanged.

Thus, stress evokes changes in PNMT gene activity too and apparently through signaling mechanisms and transcription factors associated with converging hormonal and neural activation.

CONCLUSIONS

Much remains to be revealed about the mechanisms underlying stress-regulated control of epinephrine through its biosynthesis by PNMT, and it is clear that the key to that understanding lies in identifying mechanisms controlling hormonal and neural regulated epinephrine and PNMT expression. Furthermore, hormones may play a greater role in restoring homeostasis, since they seem to maintain basal expression of epinephrine and PNMT. However, during the initial phases of stress when epinephrine is rapidly depleted or during prolonged stress where simple restorative mechanisms are insufficient, induction of PNMT to levels beyond basal expression are likely needed to provide rapid and sufficient epinephrine replenishment, and this latter situation is where neural regulatory mechanisms may come into play. We know that stress is a major contributor to psychiatric illness and major reason for smoking and nicotine addiction. Stress also places individuals at risk for cardiovascular disease, and there is high co-morbidity of psychiatric illness and cigarette smoking with cardiovascular dysfunction. Epinephrine and adrenergic function are important components for all. While linkage between these associations is currently unknown, we have hypothesized that chronic, pulsatile nicotine, as associated with smoking, may lead to hyperadrenergia so that basal epinephrine and PNMT levels are re-established at higher set points. We further propose that these changes are mediated via activation of molecular signaling cascades. Many effective treatments for a variety of illnesses seem to impact cell signaling and downstream cellular events modulating cell plasticity and neurogenesis. Identification of stress mechanisms underlying the major stress hormone, epinephrine, and associated

adrenergic responses may therefore reveal potential alternative avenues for novel treatments.

REFERENCES

Axelrod, J. (1962). Purification and properties of phenylethanolamine N-methyltransferase. *J. Biol. Chem.* **237:**1657–1660.

Axelrod, J., and Reisine, T. D. (1984). Stress hormones: Their interaction and regulation. *Science* **224:**42–49.

Berenbeim, D. M., Wong, D. L., Masover, S. J., and Ciaranello, R. D. (1979). Regulation of synthesis and degradation of rat adrenal phenylethanolamine N-methyltransferase. III. Stabilization of PNMT against thermal and tryptic degradation by S-adenosylmethionine. *Mol. Pharmacol.* **16:**482–490.

Choi, H. J., Park, S. Y., and Hwang, O. (1999). Differential involvement of PKA and PKC in regulation of catecholamine enzyme genes by PACAP. *Peptides* **20:**817–822.

Coupland, R. E., and Tomlinson, A. (1989). The development and maturation of adrenal medullary chromaffin cells of the rat *in vivo:* A descriptive and quantitative study. *Int. J. Dev. Neurosci.* **7:**419–438.

Evinger, M. J., Ernsberger, P., Regunathan, S., Joh, T. H., and Reis, D. J. (1994). A single transmitter regulates gene expression through two separate mechanisms: Cholinergic regulation of phenylethanolamine N-methyltransferase mRNA via nicotinic and muscarinic pathways. *J. Neurosci.* **14:**2106–2116.

Evinger, M. J., Towle, A. C., Park, D. H., Lee, P., and Joh, T. H. (1992). Glucocorticoids stimulate transcription of the rat phenylethanolamine *N*-methyltransferase (PNMT) gene *in vivo* and *in vitro*. *Cell. Mol. Neurobiol.* **12:**193–215.

Hwang, O., Kim, M. L., and Lee, J. D. (1994). Differential induction of gene expression of catecholamine biosynthetic enzymes and preferential increase in norepinephrine by forskolin. *Biochem. Pharmacol.* **48:**1927–1934.

Hwang, O., Park, S. Y., and Kim, K. S. (1997). Protein kinase A coordinately regulates both basal expression and cyclic AMP-mediated induction of three catecholamine-synthesizing enzyme genes. *J. Neurochem.* **68:**2241–2247.

Kvetnansky, R., Nemeths, S., Vigas, M., Oprsalova, Z., and Jurcovicova, J. (1984). Plasma catecholamines in rats during adaptation to intermittent exposure to different stressors. In Usdin, E., Kvetnansky, R., and Axelrod, J. (eds.), *Stress: The Role of Catecholamines and Other Neurotransmitters*. Gordon and Breach Science, New York, pp. 537–562.

Morita, K., Bell, R. A., Siddall, B. J., and Wong, D. L. (1996). Neural stimulation of Egr-1 messenger RNA expression in rat adrenal gland: Possible relation to phenylethanolamine N-methyltransferase gene regulation. *J. Pharmacol. Exp. Ther.* **279:**379–385.

Morita, K., and Wong, D. L. (1996). Role of Egr-1 in cholinergic stimulation of phenylethanolamine N-methyltransferase promoter. *J. Neurochem.* **67:**1344–1351.

Ross, M. E., Evinger, M. J., Hyman, S. E., Carroll, J. M., Mucke, L., Comb, M., Reis, D. J., Joh, T. H., and Goodman, H. M. (1990). Identification of a functional glucocorticoid response element in the phenylethanolamine N-methyltransferase promoter using fusion genes introduced into chromaffin cells in primary culture. *J. Neurosci.* **10:**520–530.

Sabban, E. L., and Kvetnansky, R. (2001). Stress-triggered activation of gene expression in catecholaminergic systems: Dynamics of transcriptional events. *Trends in Neuroscience* **24:**91–98.

Stachowiak, M. K., Hong, J. S., and Viveros, O. H. (1990). Coordinate and differential regulation of phenylethanolamine N-methyltransferase, tyrosine hydroxylase and proenkephalin mRNAs by neural and hormonal mechanisms in cultured bovine adrenal medullary cells. *Brain Res.* **510:**277–288.

Tai, T. C., Claycomb, R., Her, S., Bloom, A. K., and Wong, D. L. (2002). Glucocorticoid responsiveness of the rat phenylethanolamine N-methyltransferase gene. *Mol. Pharmacol.* **61:**1385–1392.

Tai, T. C., and Wong, D. L. (2002). Phenylethanolamine N-methyltransferase gene regulation by cAMP-dependent protein kinase A and protein kinase C signaling pathways. In O'Connor, D. T., and Eiden, L. E. (eds.), *The Chromaffin Cell: Transmitter Biosynthesis, Storage, Release, Actions and Informatics*. New York Academy of Sciences, New York, pp. 83–95.

Tai, T. C., and Wong, D. L. (2003). Protein kinase A and protein kinase C signaling pathway interaction in phenylethanolamine N-methytransferase gene regulation. *J. Neurochem.* **85:**816–829.

Thoenen, H., Mueller, R. A., and Axelrod, J. (1970). Neuronally dependent induction of adrenal phenylethanolamine N-methyltransferase by 6-hydroxydopamine. *Biochem. Pharmacol.* **19:**669–673.

Tonshoff, C., Hemmick, L., and Evinger, M. J. (1997). Pituitary adenylate cyclase activating polypeptide (PACAP) regulates expression of catecholamine biosynthetic enzyme genes in bovine adrenal chromaffin cells. *J. Mol. Neurosci.* **9:**127–140.

Viskupic, E., Kvetnansky, R., Sabban, E. L., Fukuhara, K., Weise, V. K., Kopin, I. J., and Schwartz, J. P. (1994). Increase in rat adrenal phenylethanolamine N-methyltransferase mRNA level caused by immobilization stress depends on intact pituitary-adrenocortical axis. *J. Neurochem.* **63:**808–814.

Wong, D. L., Anderson, L. J., and Tai, T. C. (2002a). Cholinergic and peptidergic regulation of phenylethanolamine N-methyltransferase. In O'Connor, D. T., and Eiden, L. E. (eds.), *The Chromaffin Cell: Transmitter Biosynthesis, Storage, Release, Actions and Informatics*. New York Academy of Sciences, New York, pp. 19–26.

Wong, D. L., Bildstein, C. L., Siddall, B., Lesage, A., and Yoo, Y. S. (1993). Neural regulation of phenylethanolamine N-methyltransferase in vivo: Transcriptional and translational changes. *Mol. Brain Res.* **18:**107–114.

Wong, D. L., Ebert, S. N., and Morita, K. (1996). Glucocorticoid control of phenylethanolamine N-methyltransferase gene expression: Implications for stress and disorders of the stress axis. In McCarty, R., Aguilera, G., Sabban, E., and Kvetnansky, R. (eds.), *Stress: Molecular Genetic and Neurobiological Advances*. Gordon and Breach Science, New York, pp. 677–693.

Wong, D. L., Ebert, S. N., and Morita, K. (1998a). Neural control of phenylethanolamine-N-methyltransferase via cholinergic activation of Egr-1. In Goldstein, D. S., Eisenhofer, G., and McCarty, R. (eds.), *Catecholamines: Bridging Basic Science with Clinical Medicine*. Academic Press, San Diego, pp. 77–81.

Wong, D. L., Her, S., Tai, T. C., Bell, R. A., Rusnak, M., Farkas, R., Kvetnansky, R., and Shih, J. (2002b). Stress-induced expression of phenylethanolamine N-methyltransferase: Normal and knock out animals. In McCarty, R., Aguilera, G., Sabban, E. L., and Kvetnansky, R. (eds.), *Stress: Neural, Endocrine and Molecular Studies*. Taylor and Francis, London, pp. 129–135.

Wong, D. L., Lesage, A., Siddall, B., and Funder, J. W. (1992). Glucocorticoid regulation of phenylethanolamine N-methyltransferase in vivo. *FASEB J.* **6:**3310–3315.

Wong, D. L., Siddall, B., and Wang, W. (1995). Hormonal control of rat adrenal phenylethanolamine N-methyltransferase: Enzyme activity, the final critical pathway. *Neuropsychopharmacology* **13:**223–234.

Wong, D. L., Siddall, B. J., Ebert, S. N., Bell, R. A., and Her, S. (1998b). Phenylethanolamine N-methyltransferase gene expression: Synergistic activation by Egr-1, AP-2 and the glucocorticoid receptor. *Mol. Brain Res.* **61:**154–161.

Wong, D. L., and Tai, T. C. (2002). Neural mechanisms regulating phenylethanolamine N-methyltransferase gene expression. In Nagatsu, T., Nabeshima, T., McCarty, R., and Goldstein, D. S. (eds.), *Catecholamine Research: From Molecular Insights to Clinical Medicine*. Kluwer Academic, New York, pp. 135–138.

Wong, D. L., Tai, T. C., Wong-Faull, D. C., Claycomb, R., and Kvetnansky, R. (2004). Genetic mechanisms for adrenergic control during stress. In Pacak, K., Aguilera, G., Sabban, E. L., and Kvetnansky, R. (eds.), *Stress: Current Neuroendocrine and Genetic Approaches*. New York Academy of Sciences, New York, pp. 387–397.

Wong, D. L., Zager, E. L., and Ciaranello, R. D. (1982). Effects of hypophysectomy and dexamethasone administration on central and peripheral S-adenosylmethionine levels. *J. Neurosci.* **2:**758–764.

Wurtman, R. J., and Axelrod, J. (1965). Adrenaline synthesis: Control by the pituitary gland and adrenal glucocorticoids. *Science* **150:**1464–1465.