5

EVALUATION OF NEUROTRANSMITTER STATUS IN PCOS RAT MODEL

Neurotransmitters are low-molecular weight, water soluble chemical compounds that transmit signals from one neuron to a target cell across a synapse. Neurotransmitters usually synthesized in the nerve terminal and stored in synaptic vesicles, interact with their receptors present on other neurons, muscle cells or gland cells. Due to close proximity of effector cells and well insulated presynaptic terminals, minute amounts of neurotransmitters are enough to elicit rapid and efficient responses. Once the signal is transduced, the action of neurotransmitters is rapidly terminated either through their reuptake to presynaptic terminal or via their enzymatic degradation.

Structurally, neurotransmitters can be divided into three major groups: *Monoamines* (Norepinephrine, Epinephrine, Dopamine and Serotonin); *Amino acids* (Glutamate, γ-aminobutyric acid (GABA) and Glycine) and *Peptides* (Somatostatin, Substance P, Enkephalin and Vasopressin). Of these, serotonin, norepinephrine, epinephrine, dopamine, glutamate, GABA and acetylcholine are further discussed in this chapter.

Serotonin

Serotonin (5-hydroxytryptamine; 5-HT) is the most common neurotransmitter of the peripheral system found in the enterochromafin cells of gastrointestinal tract and in blood platelets. In the central nervous system (CNS), it is mainly present in the raphe nuclei of the brain stem which project to numerous regions of the brain including the hypothalamus, limbic system, neocortex, cerebellum and the spinal cord. Although the number of neurons

containing serotonin is very less compared to others, the functional role played by serotonin is very vast owing to presence of its receptor in almost each areas of brain (Steinbush, 1981).

Serotonin is biosynthetically derived by two enzymatic steps: i) hydroxylation of tryptophan by tryptophan hydroxylase, the rate-limiting step and ii) side chain decarboxylation by 5-hydroxy tryptophan decarboxylase (aromatic amino acid decarboxylase) (Nichols & Nichols, 2008). Serotonin once synthesized, is released into the synaptic cleft, where it activates a postsynaptic receptor. There are seven classes of serotonin receptors (5-HT_1 to 5-HT_7) and all, except one (5-HT_3), are G-protein coupled receptors (GPCR) (Barrett et al., 2016). Among all the receptors, 5-HT_{1A} and 5-HT_{2A} receptors are the most studied in disease and degeneration, especially in depression, wherein down-regulation of 5-HT_{1A} and increased expression of 5-HT_{2A} has been observed (Stahl, 1994; Hung et al., 2011). 5-HT_{1A} receptors are localized in cerebral cortex, hypothalamus, hippocampus, amygdala and raphe nuclei whereas 5-HT_{2A} receptors are widely distributed in prefrontal cortex, olfactory bulb, hippocampus and basal ganglia. Both these receptors are involved in cardiovascular, pulmonary, gastrointestinal and endocrine function, sensory perception, and control of appetite, sex, sleep, mood, cognition and memory (Nichols & Nichols, 2008).

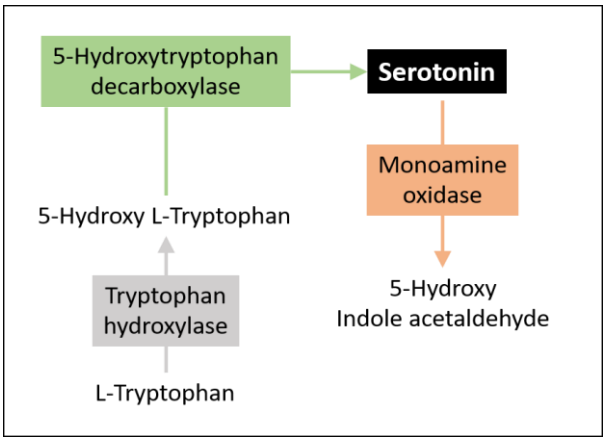

Figure 5.A: Biosynthesis and degradation of Serotonin.

The extra serotonin released into synaptic cleft is eliminated via enzymatic degradation by monoamine oxidases or its reuptake through serotonin reuptake transporter (SERT) on the presynaptic neuron (Barrett et al., 2016).

Dopamine

Dopamine is a predominant neurotransmitter in the brain, accounting for around 80% of the total catecholamine content. There are mainly four axonal pathways through which dopamine projections are spread across the brain: i) nigrostriatal pathway arises from midbrain nucleus and is involved in the control of movement; ii) mesocortical pathway originates from ventral tegmental (VTA) area and functions in learning and memory; iii) mesolimbic pathway initiates from VTA and is involved in pleasure and reward stimuli; iv) tuberoinfundibular pathway projects from periventricular and arcuate nuclei of hypothalamus and influences anterior pituitary functions (Vallone et al., 2000).

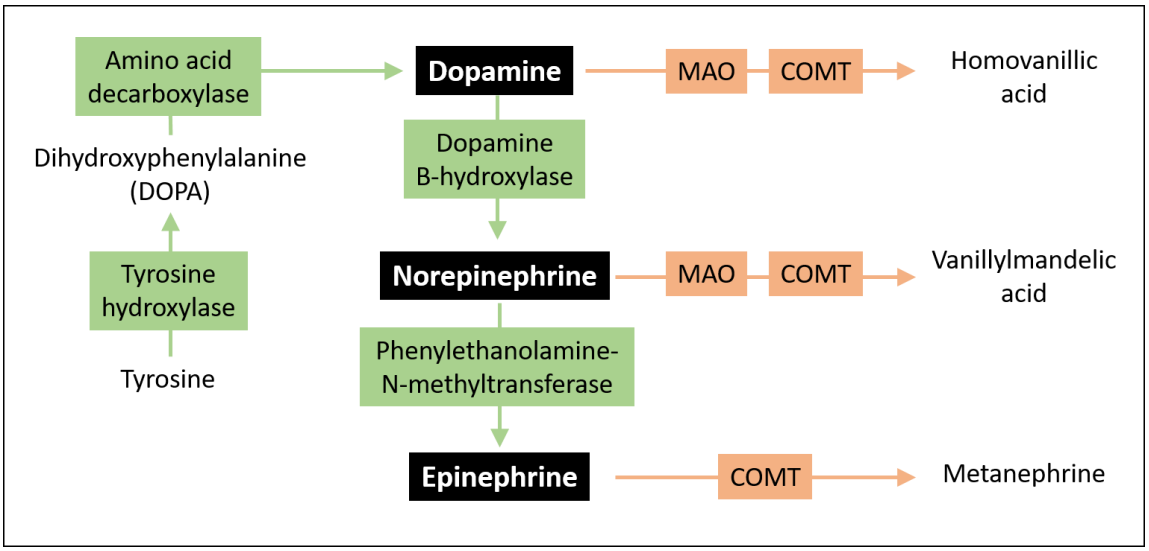

Figure 5.B: Biosynthesis and degradation of Catecholamines

Dopamine is synthesized from precursor amino acid-tyrosine through two enzymes – tyrosine hydroxylase (a rate-limiting enzyme) and DOPA-decarboxylase. The released dopamine in synaptic cleft is taken up by dopamine transporter or is metabolized to inactive compounds by monoamine oxidase (MAO) and catechol-*O*-methyl transferase (COMT) (Barrett et al., 2016). Dopamine exerts its effect through binding to its receptors which are mainly of five types (D_1 to D_5), all belonging to the GPCR family. D_1 and D_2 receptors are the most widely expressed receptors of dopamine. D_1 receptor is largely expressed in nucleus accumbens, cerebral cortex, and amygdala. D_2 receptor is an autoreceptor and predominantly found in the striatum, nucleus accumbens, hypothalamus, cortical areas, amygdala, hippocampus and pituitary gland (Beaulieu & Gainetdinov, 2011). D_1 and D_2 receptors synergistically regulate learning and memory, locomotor activity, vasodilation, and reward behaviour, while D_2

receptor alone mediates hormone secretion from pituitary, adrenal aldosterone release and sympathetic activity (Missale et al., 1998; Beaulieu & Gainetdinov, 2011).

Norepinephrine & Epinephrine

Norepinephrine and epinephrine are the catecholamines, best known for their 'flight or fight' response that is mediated by activation of sympathetic nervous system. Norepinephrine-containing neurons are principally found in the locus coeruleus of pons and innervate paraventricular, supraoptic and periventricular nuclei of the hypothalamus, thalamus, telencephalon, neocortex, cerebellum and spinal cord (Barrett et al., 2016).

Catecholamines – dopamine, norepinephrine and epinephrine are synthesized by the same enzymatic pathway using tyrosine or phenylalanine as the precursor. In noradrenergic neurons, dopamine formed by tyrosine hydroxylase and dopa-decarboxylase reaction is transported into synaptic vesicle and converted to norepinephrine via dopamine-β-hydroxylase enzyme. Adrenergic neurons contain the enzyme phenylethanolamine-*N*-methyltransferase (PNMT) which catalyses the conversion of norepinephrine into epinephrine (Barrett et al., 2016). Norepinephrine released in synaptic cleft has three main fates – it binds to postsynaptic or presynaptic receptor or is taken up by norepinephrine reuptake transporter (NET) or is enzymatically degraded via MAO or COMT (Barrett et al., 2016).

Epinephrine and norepinephrine both act on α- and β-adrenergic receptors (adrenoceptors), with norepinephrine having a greater affinity for α-adrenoceptors and epinephrine for β-adrenoceptors (Barrett et al., 2016). All these receptors are GPCRs. α_1-adrenergic receptors (α_1-AR) are most commonly present in prefrontal cortex, dorsal raphe, reticular thalamic nucleus and neocortex, where they aid in functions such as arousal, attention, motivation, locomotion and memory formation (Piascik & Perez, 2001). β-adrenergic receptors are mostly located outside the CNS in heart, kidney, smooth and skeletal muscles and adipose tissue and mediate flight-fight response through increasing cardiac output, glucose uptake and muscle contraction (Piascik & Perez, 2001).

Glutamate

Glutamate is the most important excitatory neurotransmitter of the mammalian central nervous system (CNS), playing an important role in memory, synaptic plasticity and neuronal development. However, glutamate overstimulation is also implicated in neurodegeneration

(Ribeiro et al., 2017). Glutamate is synthesized through two different pathways: i) transamination of α-ketoglutarate into glutamate via the enzyme GABA-transaminase (GABA-T); ii) conversion of glutamine to glutamate by glutaminase enzyme. Glutamate reuptake transporters located on glutamatergic neurons as well as on glial cells, maintain glutamate levels in synapse. Also, excess glutamate can be eliminated through enzymatic conversion by glutamate dehydrogenase.

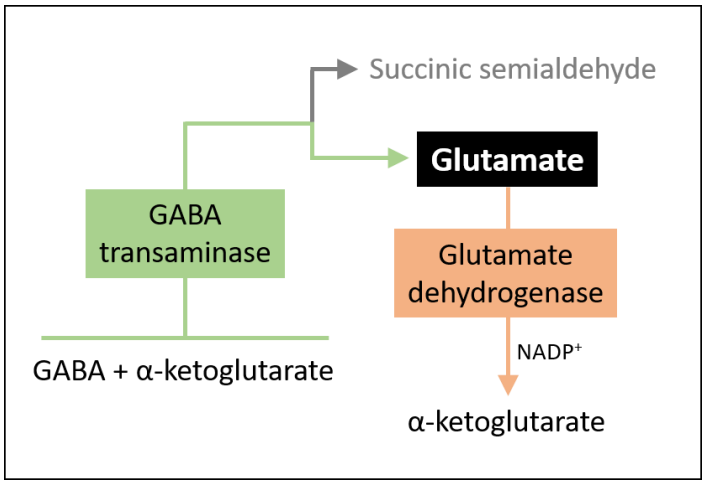

Figure 5.C: Biosynthesis and degradation of Glutamate

There are mainly two types of glutamate receptors – ionotropic and metabotropic receptors. Ionotropic glutamate receptors are fast-acting glutamate receptors that can be subdivided into AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionate), Kainate and NMDA (N-methyl-D-aspartate) receptors and predominantly function in long-term potentiation, and learning and memory. Metabotropic glutamate receptors have eight GPCR subtypes ($mGluR_1$ to $mGluR_8$) widely distributed in cerebral cortex, hippocampus, olfactory bulb and nucleus accumbens and play roles in synaptic plasticity, motor function and learning (Barrett et al., 2016; Ribeiro et al., 2017).

γ-amino-butyric acid (GABA)

GABA is the major inhibitory mediator in the brain and mediates both presynaptic and postsynaptic inhibition. Being an inhibitory current, it influences most of the body functions including sleep-wake cycle, learning and memory, pain response, hormone secretion and reproductive behaviour. GABA is synthesized from decarboxylation of glutamate, the reaction catalysed by the enzyme glutamate decarboxylase. Excess GABA is eliminated through GABA-transaminase (GABA-T) enzyme, which converts GABA into succinic

semialdehyde and then to succinate in the citric acid cycle. In addition, there is an active reuptake of GABA via the GABA transporter (Barrett et al., 2016).

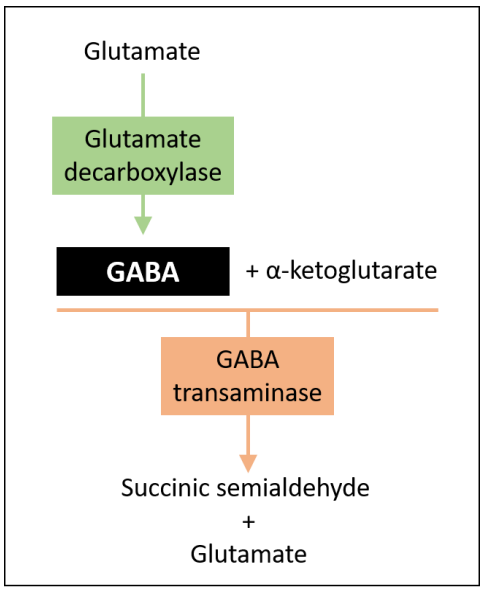

Figure 5.D: Biosynthesis and degradation of GABA

GABA receptors are found with three different subtypes – $GABA_A$, $GABA_B$ and $GABA_C$. The $GABA_A$ and $GABA_B$ receptors are widely distributed in the CNS, whereas in adult vertebrates the $GABA_C$ receptors are found almost exclusively in the retina (Barrett et al., 2016). $GABA_A$ and $GABA_C$ are fast-acting ionotropic receptors while $GABA_B$ receptors are G-protein-coupled, metabotropic receptor (Barrett et al., 2016). $GABA_B$ receptors are predominantly localized to cerebral cortex, cerebellum, hippocampus, hypothalamus, and amygdala and they are found on both pre- and postsynaptic neurons (Benarroch, 2012).

Acetylcholine (ACh)

Acetylcholine (ACh) is the transmitter at neuromuscular junctions, in autonomic ganglia and in postganglionic parasympathetic nerve-target organ junctions and some postganglionic sympathetic nerve-target junctions (Barrett et al., 2016). Acetylcholine is mainly localised to septal nuclei and nucleus basalis of the basal forebrain complex and to the pontomesencephalotegmental complex. The cholinergic neurons of basal forebrain complex projects to the hippocampus and neocortex, whereas that of pontomesencephalotegmental complex innervates dorsal thalamus and forebrain regions. The major functions of acetylcholine include muscle contraction, arousal, learning and memory, reward, motor control and analgesia. Biosynthesis of acetylcholine occurs through the enzyme choline

acetyltransferase (ChAT) from the substrates choline and acetyl-CoA. Acetylcholine formed is stored in the synaptic vesicle and released into synapse upon stimulation. The excess acetylcholine is removed from the synaptic cleft through the enzyme acetylcholinesterase (AChE).

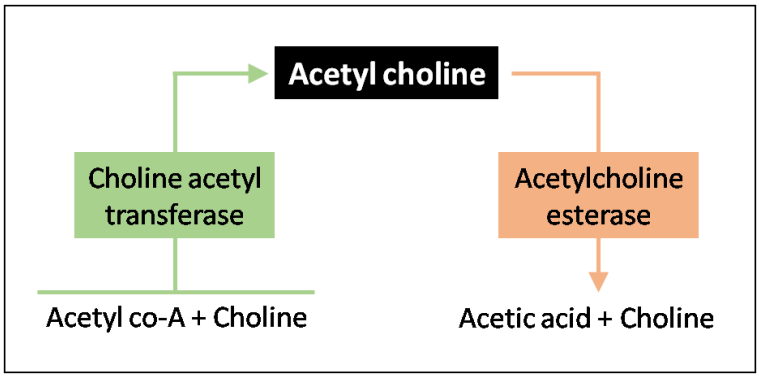

Figure 5.E: Biosynthesis and degradation of Acetyl choline

Acetylcholine signals through two classes of receptors – metabotropic muscarinic receptors (mAChRs) and ionotropic nicotinic receptors (nAChRs) (Picciotto et al., 2012). Depending upon their location, nicotinic acetylcholine receptors can be termed as N_M (found at neuromuscular junction) and N_N (found in CNS and autonomic ganglia). In brain, the nAChRs regulate the release of both excitatory and inhibitory neurotransmitters and hence, can modulate various body functions (Zoli et al., 2015). Muscarinic receptors are subdivided into five classes – M_1 to M_5. They are also present pre- and postsynaptically throughout the brain and can act as autoreceptors for other neurotransmitter release; for example, stimulation of presynaptic M_2/M_4 mAChRs reduces glutamate release from corticocortical and corticostriatal synapses, whereas M_1/M_5 mAChRs can stimulate dopamine release from striatal synaptosomes (Picciotto et al., 2012).

Rationale

It is thus clear from literature that neurotransmitters regulate various body functions such as motor control, cognition, learning and memory, sleep-wake cycle, appetite, mood and hormone secretion. In addition, various reports indicate the role of these neurotransmitters in regulation of reproductive axis (Smith & Jennes, 2001; Hrabovszky & Liposits, 2013; Bhattarai et al., 2014). In situ hybridization and immunohistochemical studies have identified presence of several neurotransmitter-containing neurons in the GnRH neurons of hypothalamus, suggesting a direct influence of these neurotransmitters in GnRH release

(Hrabovszky & Liposits, 2013). Furthermore, studies implicate that neurotransmitters interact with estrogen and progesterone receptors and facilitate steroid-mediated feedback regulation of HPO axis (Christian et al., 2009; Barth et al., 2015).

In this regard, reports have indicated stimulatory as well as inhibitory effect of norepinephrine on LH release through activation of α_1- and β-adrenergic receptors, respectively (Weesner et al., 1993; Clarke & Pompolo, 2005). Also, it was evident that norepinephrine can influence the amplitude, frequency as well as transcription of both GnRH and LH (Smith & Jennes, 2001). Similarly, the role of serotonin in GnRH release is contradictory but a recent study has described that the stimulatory effect of serotonin on GnRH is through $5HT_{2A}$ receptor whereas $5HT_{1A}$ receptor is responsible for serotonin-mediated GnRH inhibition (Bhattarai et al., 2014). Dopamine is a potent inhibitor of GnRH and gonadotropin release and this action is mediated via D_1 and D_2 dopamine receptors. Dopamine can also inhibit transcription of gonadotropin α and β-subunits, as evident by experiments on LβT2-gonadotroph cell line (Henderson et al., 2008; Liu and Herbison, 2013). Along with dopamine, acetylcholine also influences GnRH secretion whereby activation of nicotinic ACh receptor results into increased GnRH release, while muscarinic receptor suppresses GnRH action (Krsmanovic et al., 1998). Glutamate and GABA are the major excitatory and inhibitory neurotransmitters, respectively for GnRH release. Glutamate receptors AMPA, NMDA are Kainate and are co-localized with GnRH neurons of hypothalamus. Electrophysiological and calcium imaging studies have demonstrated direct influence of glutamate in GnRH excitability and firing pattern (Iremonger et al., 2010). In contrast to glutamate, evidence of direct inhibitory action of GABA on GnRH release has been observed. This inhibition of GABA is achieved through both the $GABA_A$ and $GABA_B$ receptors; however, $GABA_B$ is more explored in terms of GnRH regulation (Catalano et al., 2010). Also, binding of GABA to $GABA_{A/C}$ can increase gonadoropin release from pituitary, whereas $GABA_B$ receptor activation inhibits LH pulsatility (Virmani et al., 1990). In addition to their individual effects, glutamate and GABA interact with various neurotransmitters and influence GnRH release (Liu and Herbison, 2013). It is thus clear that neurotransmitters directly or indirectly modulate expression as well as activity of both the GnRH and gonadotropin from hypothalamus and pituitary respectively.

As mentioned in the introduction (Chapter 1), PCOS is a disorder underpinning neuroendocrine abnormalities, characterized by increased GnRH and LH/FSH ratio. In line

with this, the present study has demonstrated increased GnRH mRNA expression in letrozole-induced PCOS rat model. Also, the PCOS rats exhibited increased expression of FSH and LH transcripts in pituitary which lead to elevated serum LH/FSH ratio as shown in Chapter 3. As indicated in the above-cited literature, neurotransmitters affect expression and activity of GnRH as well as LH and FSH. In this context, various studies have reported low levels of norepinephrine, dopamine and serotonin in serum of PCOS women (Shi et al., 2011). Also, catecholamine levels were altered in PCOS women follicular fluid (Musali et al., 2016). Reports also indicate that low dopaminergic tone may be responsible for increased LH pulsatility in PCOS (Kalro et al., 2001; Gomez et al., 2011). All these studies indicate a putative role of neurotransmitters in neuroendocrine aberrations of PCOS. However, due to limitation of availability of human samples and concerned ethical issues, the detailed understanding of neurotransmitter function in PCOS condition is lacking. Thereby, an objective of the current study was to evaluate the status of GnRH regulatory neurotransmitters in PCOS condition, using the rat model.

Materials and Methods

Letrozole induced PCOS rat model was developed as mentioned in the materials and methods section (Chapter 2). Following the validation, PCOS positive animals were sacrificed by decapitation and tissues (Hypothalamus, Pituitary, Hippocampus, Frontal cortex, Ovaries and Adrenal glands) were dissected out. Tissues were immediately processed for estimations or they were stored at -80 °C until use. Neurotransmitters (5-HT, NE, DA, Glut, GABA) were estimated using HPLC method while for epinephrine estimation, spectrophotometric method was employed (Chapter 2). Status of neurotransmitters depends upon their synthesis and enzymatic degradation. Hence, activities of neurotransmitter synthesizing and metabolizing enzymes were measured. For synthesis of serotonin and GABA, activities of tryptophan decarboxylase (TDC) and glutamic acid decarboxylase (GAD) were measured spectroflourimetrically. For the estimation of GABA-transaminase (GABA-T) activity, a kinetic method was used. Most of the monoamine neurotransmitters including serotonin, norepinephrine, dopamine and GABA are degraded through monoamine oxidase (MAO) enzyme. The activity of MAOs, glutamate dehydrogenase (GDH) and acetylcholine esterase (AChE) was measured using kinetic method. Tyrosine hydroxylase and catechol-*O*-methyl transferase (COMT) are the important enzymes for catecholamine synthesis and metabolism respectively, however, since estimation of their activity was not feasible in the present study, mRNA expression studies of these two enzymes were performed.

Each neurotransmitter has numerous receptors through which it affects various biological processes. As described in earlier section, the receptor of neurotransmitter that is profoundly implicated in reproductive processes, has been accounted for the present study. Expression profile of the neurotransmitter receptors- 5-HT_{1A}-serotonin receptor, D_2 dopamine receptor, α_1-adrenergic receptor, NMDA-glutamate receptor, $GBABA_{B1}$ receptor, and M_2-Muscarinic acetylcholine receptor was carried out using quantitative real-time PCR.

RESULTS

Neurotransmitter levels in PCOS rats

Neurotransmitters serotonin, norepinephrine, dopamine, glutamate and GABA were estimated using a high sensitivity electrochemical detector coupled with HPLC. The levels of these neurotransmitters in different tissues are demonstrated in Figures 5.1 and 5.2. Hypothalamus of PCOS rats exhibited a significant decrease in the levels of 5-HT ($P<0.001$), NE ($P<0.001$), Epi ($P<0.01$), DA ($P<0.01$) and GABA ($P<0.001$), while a marked increase was observed in glutamate levels ($P<0.001$) as compared to control animals. Similar trend was also observed in pituitary of PCOS rats as seen in the case of hypothalamus wherein reduction in 5-HT ($P<0.001$), NE ($P<0.01$), Epi ($P<0.05$), DA ($P<0.01$) and GABA ($P<0.001$) content was seen with elevated levels of glutamate ($P<0.001$) compared to pituitary of control rats. When analysed for neurotransmitter levels in hippocampus and frontal cortex, no difference was observed in Epi content between control and PCOS animals. However, decreased levels of 5-HT ($P<0.01$), NE ($P<0.01$), DA ($P<0.05$) and GABA ($P<0.01$) whilst higher levels of glutamate ($P<0.01$) were found in hippocampus region of PCOS rat brain as compared to control animals. Frontal cortex demonstrated similar tendency in the neurotransmitter levels as observed in the hippocampus. Ovary is the site wherein locally synthesized neurotransmitters aid in follicular development and maturation while adrenal gland elicits a stress response through the release of sympathetic neurotransmitters. Neurotransmitter profile of ovary was quite different from other tissues, wherein reduced levels of 5-HT ($P<0.05$) and DA ($P<0.05$) and increased NE ($P<0.05$) and Epi ($P<0.05$) content was observed in PCOS, with no change in GABA and glutamate levels. Similar to ovary, significantly elevated NE ($P<0.01$) and Epi ($P<0.05$) was observed in adrenal gland of PCOS animals with no change in other neurotransmitters.

In order to rule out any direct effect of letrozole on neurotransmitter levels, neurotransmitters were also estimated in testosterone propionate (TP)-induced PCOS rat model (Chaudhari & Nampoothiri, 2017). TP-induced animals demonstrated similar neurotransmitter profile as observed for the letrozole-induced PCOS rats, indicating that the observed neurotransmitter alteration is a result of PCOS and not due to any artefact of drug administration.

Neurotransmitter synthesizing enzymes in PCOS rats

The content of any compound depends upon the rate of its synthesis and degradation. The activity of neurotransmitter synthesizing enzymes was measured in PCOS animals (Figures 5.3 and 5.4).

Serotonin is synthesized by the enzyme tryptophan decarboxylase (TDC), which utilizes 5-hydroxytryptophan as a substrate. The activity of TDC was reduced in all the brain tissues analysed of PCOS animals ($P<0.01$), except in the hippocampus. There was also no change in TDC activity between control and PCOS animals for ovary and adrenal tissues. GABA-Transaminase (GABA-T) utilizes α-ketoglutarate and GABA and converts them into glutamate and succinic semialdehyde. Thus, GABA-T acts as a synthesizing enzyme for glutamate and metabolizing enzyme for GABA. GABA-T activity demonstrated marked increase in hypothalamus ($P<0.01$), pituitary ($P<0.01$), hippocampus ($P<0.05$) and frontal cortex ($P<0.05$) with no change in ovary and adrenal gland of PCOS rats as compared to control animals. Glutamic acid decarboxylase (GAD) enzyme catalyses the conversion of glutamate into GABA. When analysed for GAD activity, only hypothalamus ($P<0.01$) and pituitary ($P<0.01$) exhibited notable decrease in GAD activity whereas other tissues did not show any change in the GAD activity. Furthermore, tyrosine hydroxylase (TH) is the rate-limiting enzyme for all catecholamine synthesis. Tyrosine hydroxylase mRNA transcripts were reduced in hypothalamus ($P<0.01$) and pituitary ($P<0.05$) whereas up-regulated expression of TH was observed in PCOS ovary ($P<0.05$) while no change was observed in other tissues studied.

Neurotransmitter metabolizing enzymes in PCOS rats

Neurotransmitters are quickly metabolized through neurotransmitter degrading enzymes which are secreted into the synaptic cleft.

Majority of the neurotransmitters such as serotonin, norepinephrine, dopamine and epinephrine are metabolized through Monoamine oxidase (MAO) enzyme. MAO is mainly

found in two isoforms – A & B, of which MAO-A is predominantly localized to central nervous system and MAO-B is found in the peripheral system. Results are depicted in Figures 5.5 and 5.6. To our surprise, a significant increase in MAO-A was observed in hypothalamus ($P<0.001$), pituitary ($P<0.001$), hippocampus ($P<0.01$), frontal cortex ($P<0.01$), with no change in ovary and adrenal gland of PCOS rats as compared to control animals. MAO-B also followed a similar trend in all the tissues but it was less obvious as compared to MAO-A activity. Another major enzyme which degrades catecholamines-dopamine, norepinephrine and epinephrine is Catechol-*O*-methyl transferase (COMT). Due to unavailability of the co-factor, *S*-adenosyl methionine (SAM), COMT activity was not measured and instead quantification of its mRNA was carried out in PCOS condition. The transcript of *COMT* was markedly elevated in hypothalamus ($P<0.01$) and pituitary ($P<0.05$) of PCOS rats while no change was observed in other tissues. In addition, metabolizing enzymes glutamate dehydrogenase (GDH) and acetylcholine esterase (AChE) that degrades glutamate and acetylcholine, respectively were also assessed. Acetylcholine esterase activity was also higher in PCOS animals as evident in hypothalamus ($P<0.01$), pituitary ($P<0.01$) and hippocampus ($P<0.05$) whereas no difference was observed in the activity of AChE in frontal cortex, ovary and adrenal gland. In contrast to other metabolizing enzymes activity, that is increased in PCOS animals, GDH enzyme activity was significantly reduced in hypothalamus ($P<0.01$), pituitary ($P<0.01$), hippocampus ($P<0.05$) and frontal cortex while no change in adrenal gland GDH activity was observed as compared to control animals.

Neurotransmitter receptor profile in PCOS rats

Neurotransmitters bind to their receptors on pre- or postsynaptic neurons and relay their biological action. Consequently, an mRNA expression profile of various neurotransmitter receptors was generated using quantitative real-time PCR technique (Figures 5.7 and 5.8). The hypothalamus of PCOS rats demonstrated a profound reduction in transcript levels of $5HT_{1A}$ ($P<0.001$), α_1-adrenergic receptor ($P<0.05$), D_2-dopamine receptor ($P<0.05$), $GABA_{B1}$ ($P<0.01$) and M_2-muscarinic acetylcholine receptor ($P<0.05$) while the expression of NMDA-glutamate receptor ($P<0.01$) was significantly increased in PCOS animals as compared to controls. Similar tendency in expression profile was also observed in pituitary of PCOS animals with more profound decrease in $GABA_{B1}$ ($P<0.001$) and increased expression of *NMDAR* ($P<0.001$) transcript as compared to hypothalamic alterations. Hippocampus and frontal cortex of PCOS rats demonstrated down-regulation of $5HT_{1A}$ ($P<0.01$), α1-adrenergic receptor ($P<0.05$), D_2-dopamine receptor ($P<0.05$) and $GABA_{B1}$ ($P<0.01$) receptor and

increased expression of NMDA-glutamate receptor ($P<0.05$), whereas no difference was observed in expression of M_2-muscarinic acetylcholine receptor of control and PCOS animals. The expression profile of ovarian transcripts demonstrated reduced expression of *5HT$_{1A}$* ($P<0.01$), and D_2-dopamine receptor ($P<0.05$) whereas α_1-adrenergic receptor ($P<0.05$) expression was significantly elevated in PCOS condition with no notable change in other receptor expression. When analysed for adrenal gland transcripts, significant increase was observed in only *NMDA* receptor expression of PCOS animals ($P<0.05$), while no change was observed in the transcripts of other neurotransmitter receptors.

In summary, current data demonstrates an apparent change in the neurotransmitter status in PCOS condition. Also, the decrease in contents of neurotransmitters content is mainly mediated due to their increased degradation. Further, the change in expression of the neurotransmitter receptors is more profound in hypothalamus, pituitary and ovary of PCOS animals suggesting a direct role of these neurotransmitters in ameliorating the functions of reproductive axis. However, further downstream effects of altered receptor expression with HPO axis function needs to be studied.

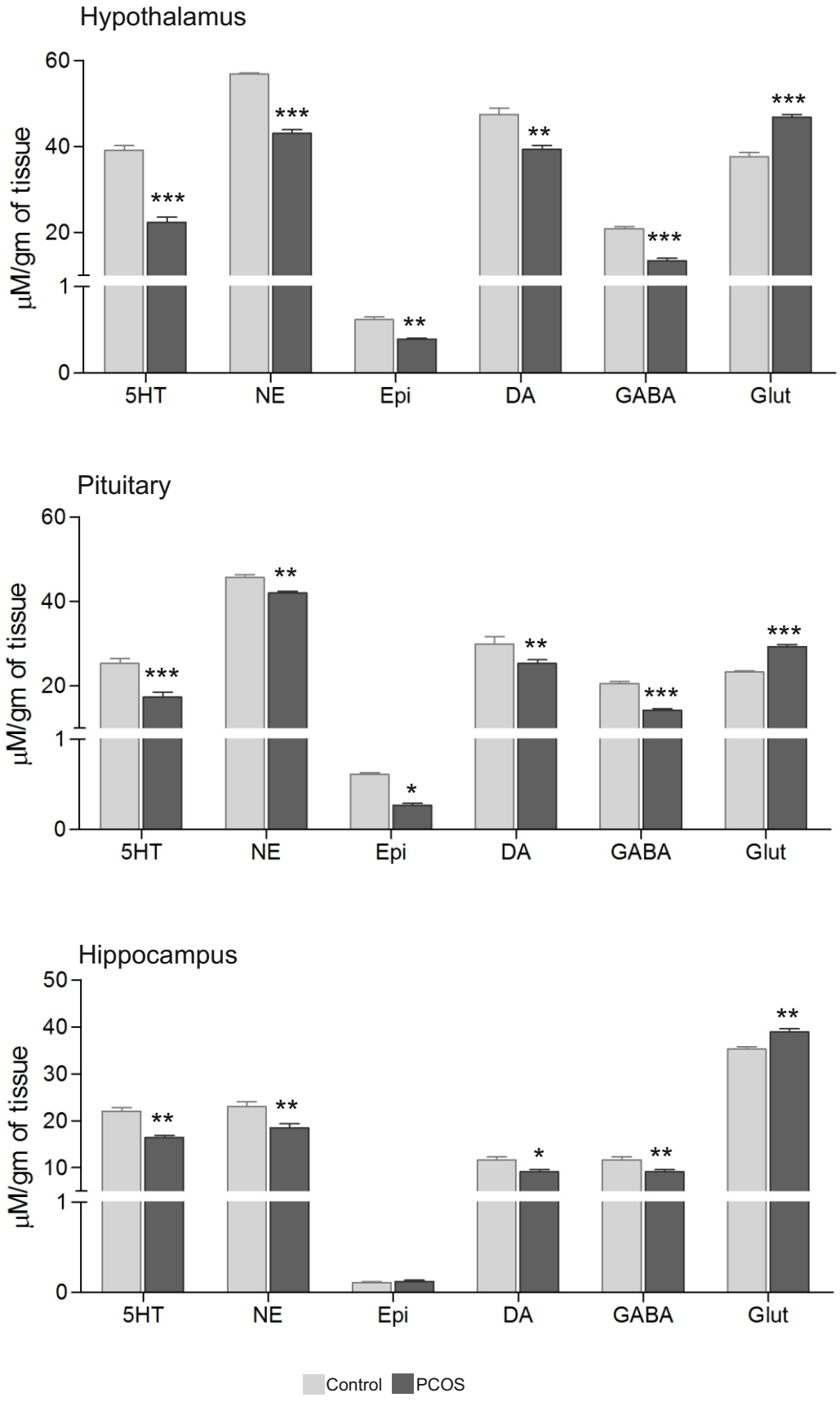

Figure 5.1: Neurotransmittter levels in control and PCOS animals. Error bars represent SEM; N=6 per group. *P<0.05; **P<0.01; ***P<0.001 as compared to control group.

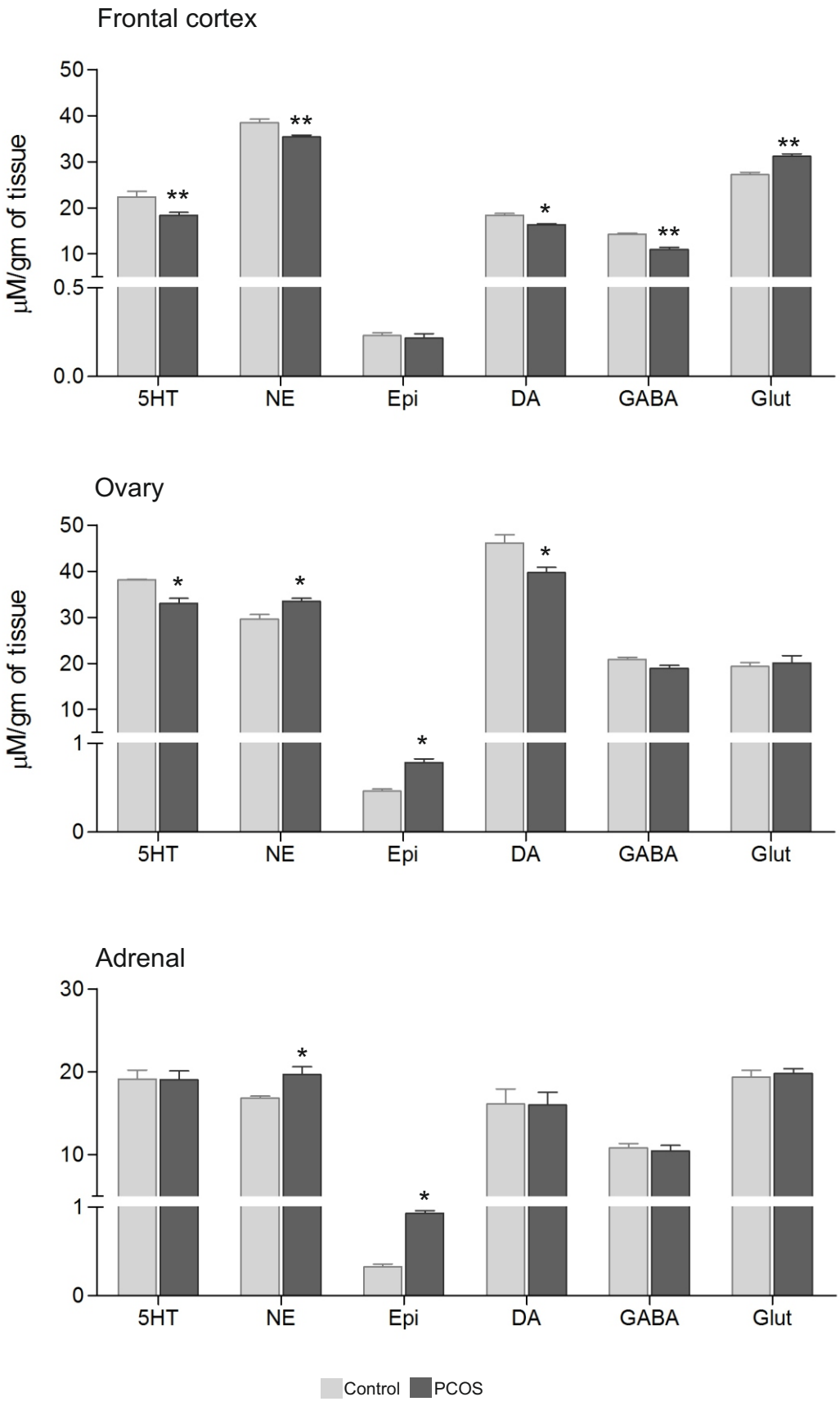

Figure 5.2: Neurotransmittter levels in control and PCOS animals. Error bars represent SEM; N=6 per group. *P<0.05; **P<0.01; ***P<0.001 as compared to control group.

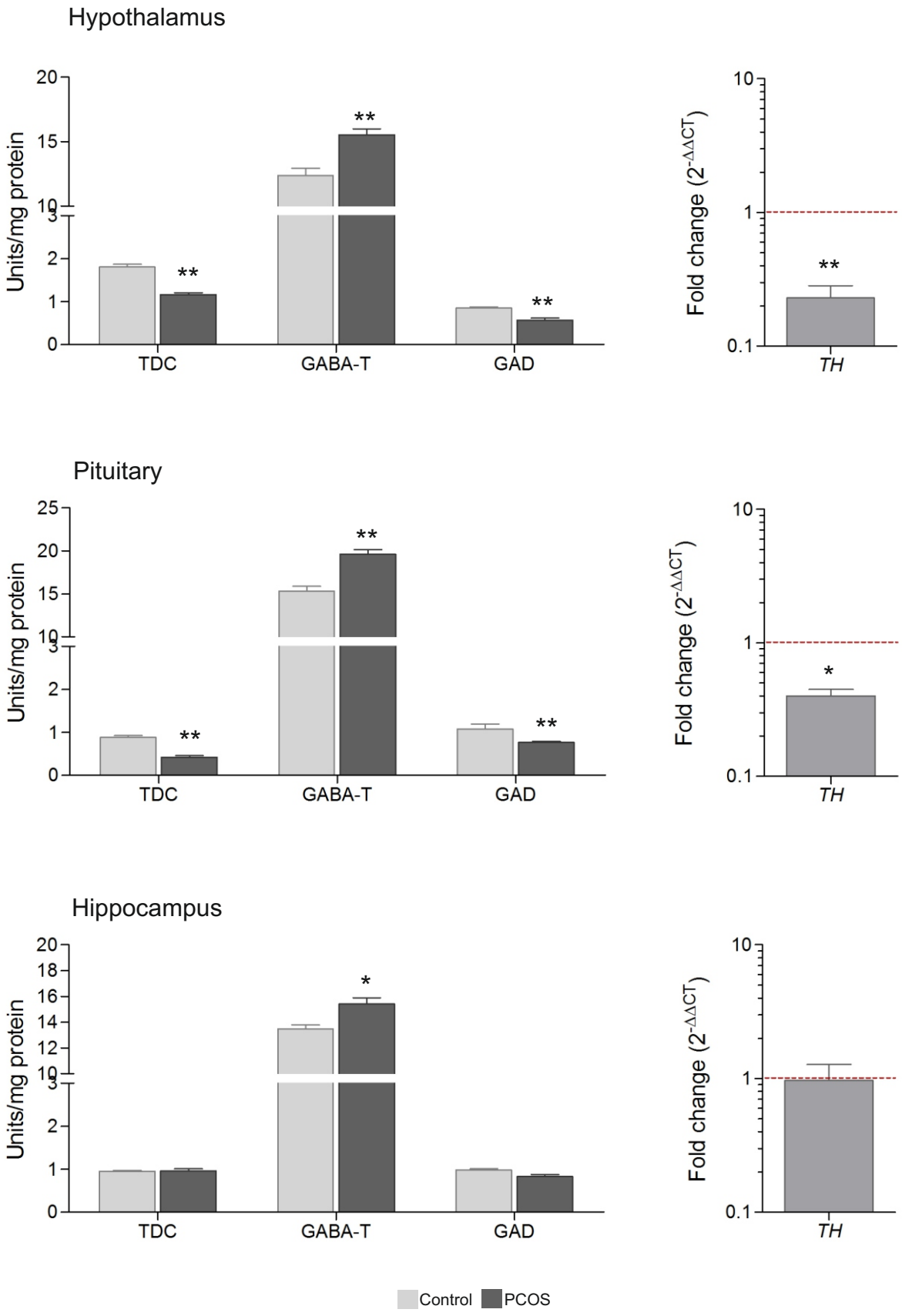

Figure 5.3: Status of neurotransmitter synthesizing enzymes. Left: Activity of enzymes. Right: Transcript levels . Values are mean fold change in gene expression of PCOS group samples as compared to control samples (represented by red dashed line). Error bars represent SEM; N=6 per group. *P<0.05; **P<0.01; ***P<0.001 as compared to control group.

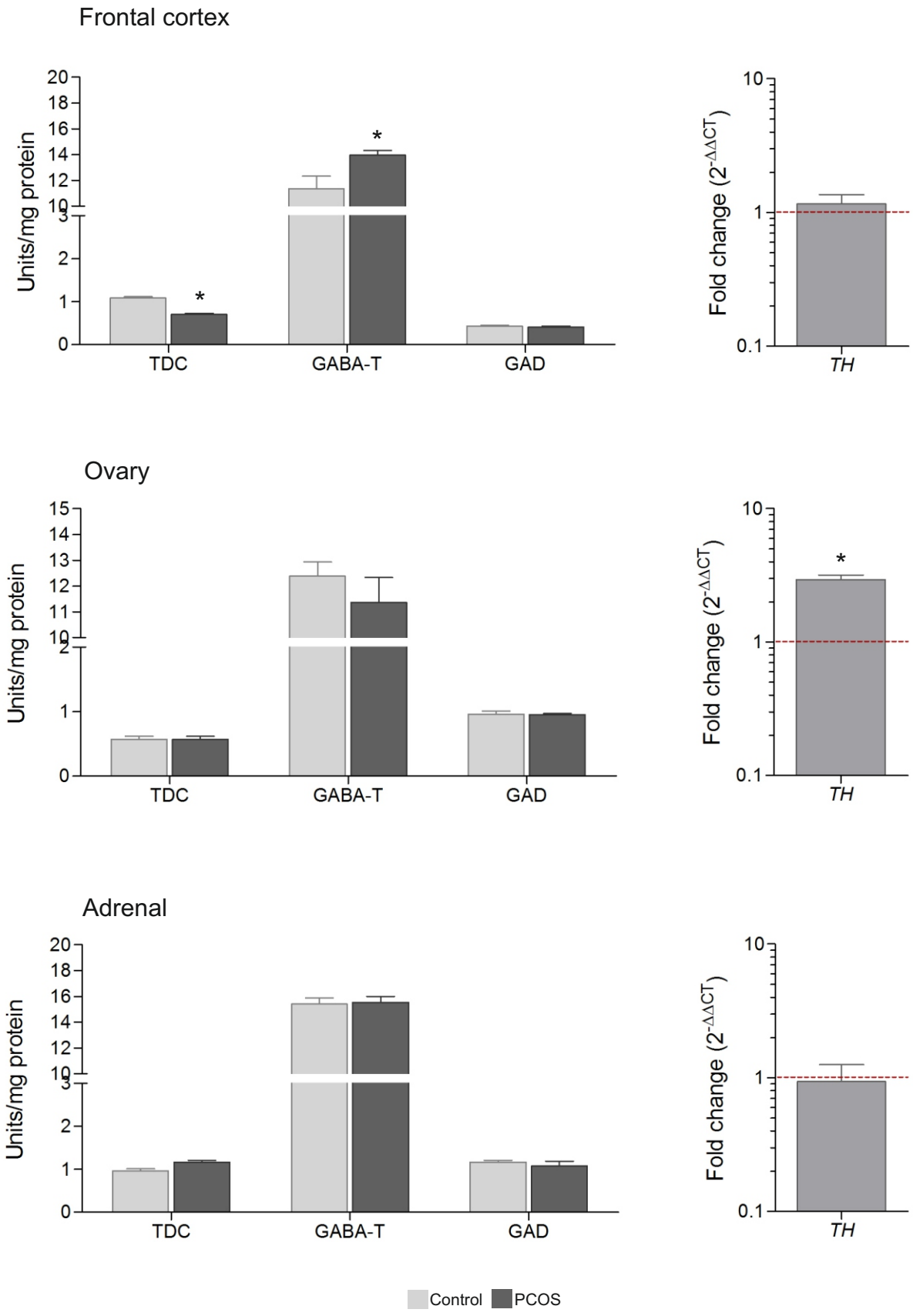

Figure 5.4: Status of neurotransmitter synthesizing enzymes. Left: Activity of enzymes. Right: Transcript levels . Values are mean fold change in gene expression of PCOS group samples as compared to control samples (represented by red dashed line). Error bars represent SEM; N=6 per group. *P<0.05; **P<0.01; ***P<0.001 as compared to control group.

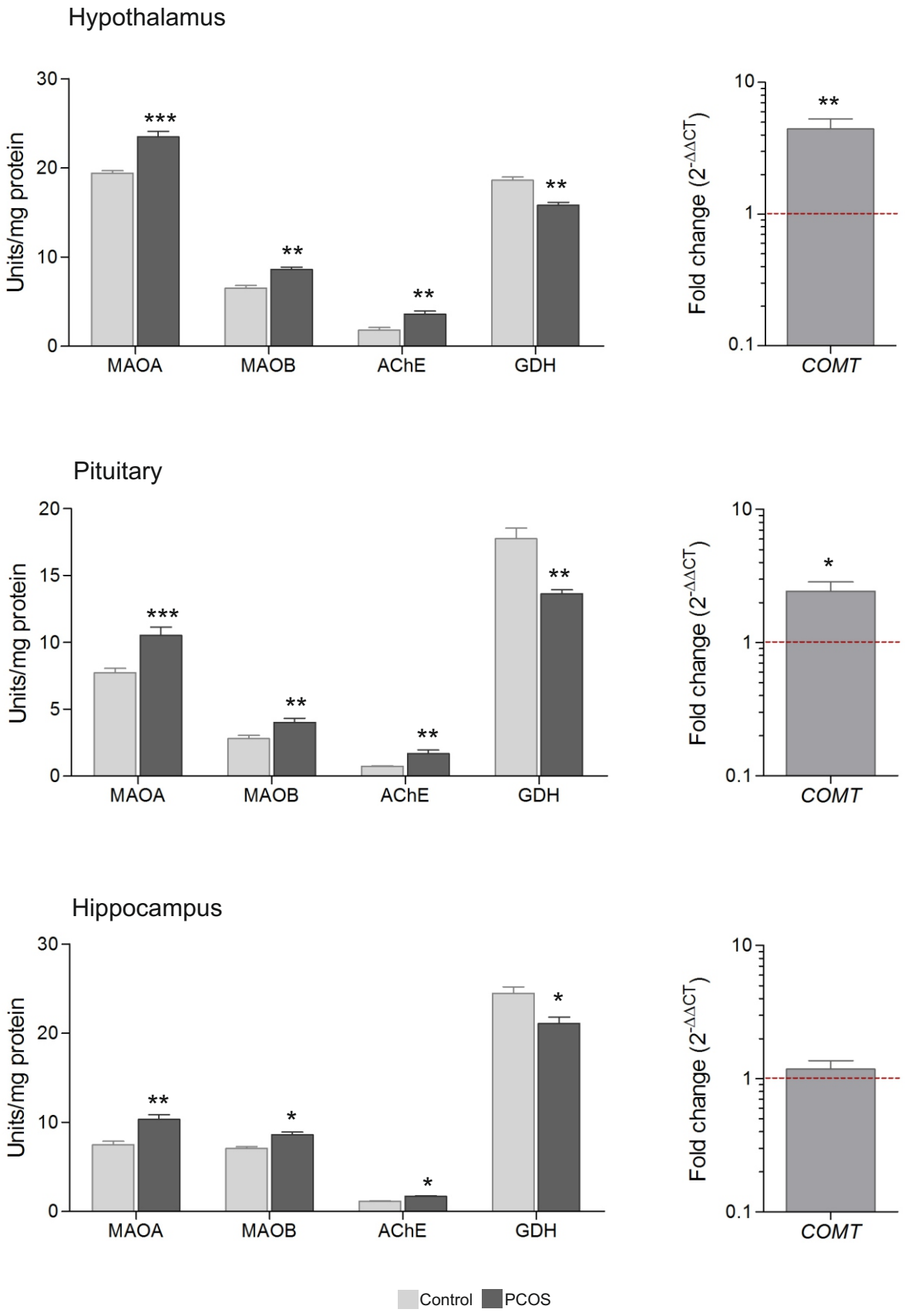

Figure 5.5: Status of neurotransmitter degrading enzymes. Left: Activity of enzymes. Right: Transcript levels . Values are mean fold change in gene expression of PCOS group samples as compared to control samples (represented by red dashed line). Error bars represent SEM; N=6 per group. *P<0.05; **P<0.01; ***P<0.001 as compared to control group.

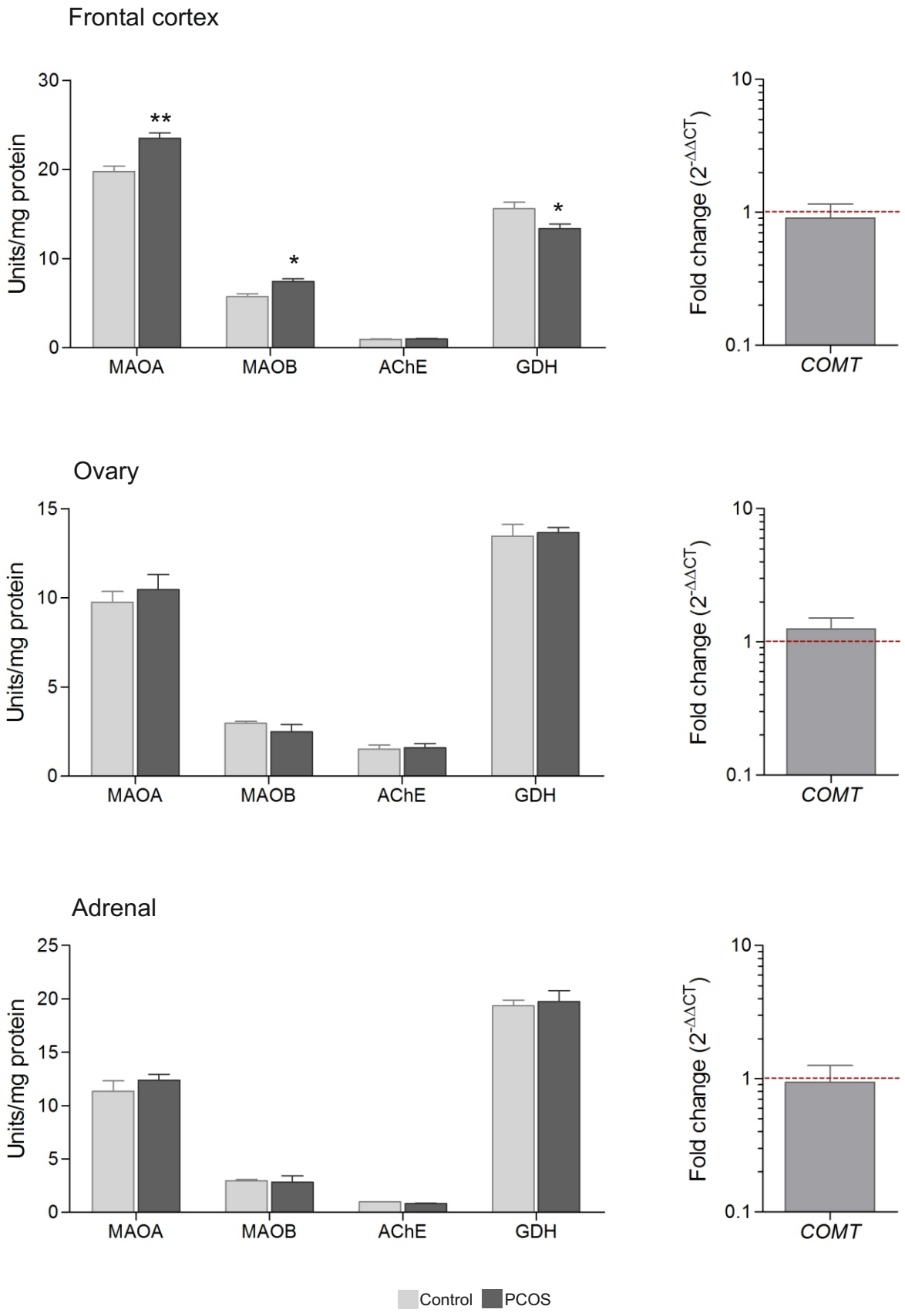

Figure 5.6: Status of neurotransmitter degrading enzymes. Left: Activity of enzymes. Right: Transcript levels . Values are mean fold change in gene expression of PCOS group samples as compared to control samples (represented by red dashed line). Error bars represent SEM; N=6 per group. *P<0.05; **P<0.01; ***P<0.001 as compared to control group.

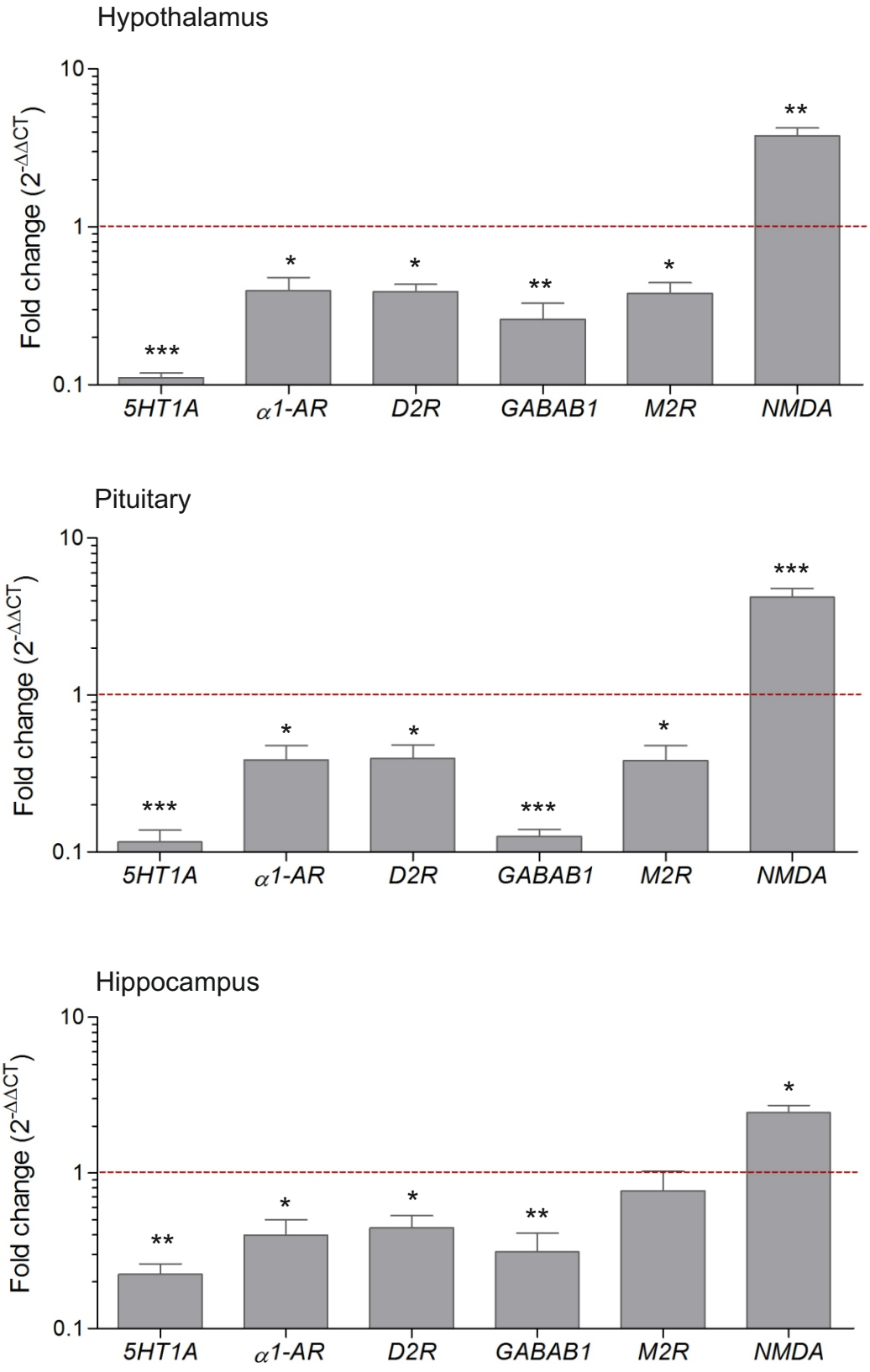

Figure 5.7: Relative gene expression of neurotransmitter receptors. Values are mean fold change in gene expression of PCOS group samples as compared to control samples (represented by red dashed line). ACTH and corticosterone levels in serum. Error bars represent SEM; N=6 per group. *P<0.05; **P<0.01; ***P<0.001 as compared to control group.

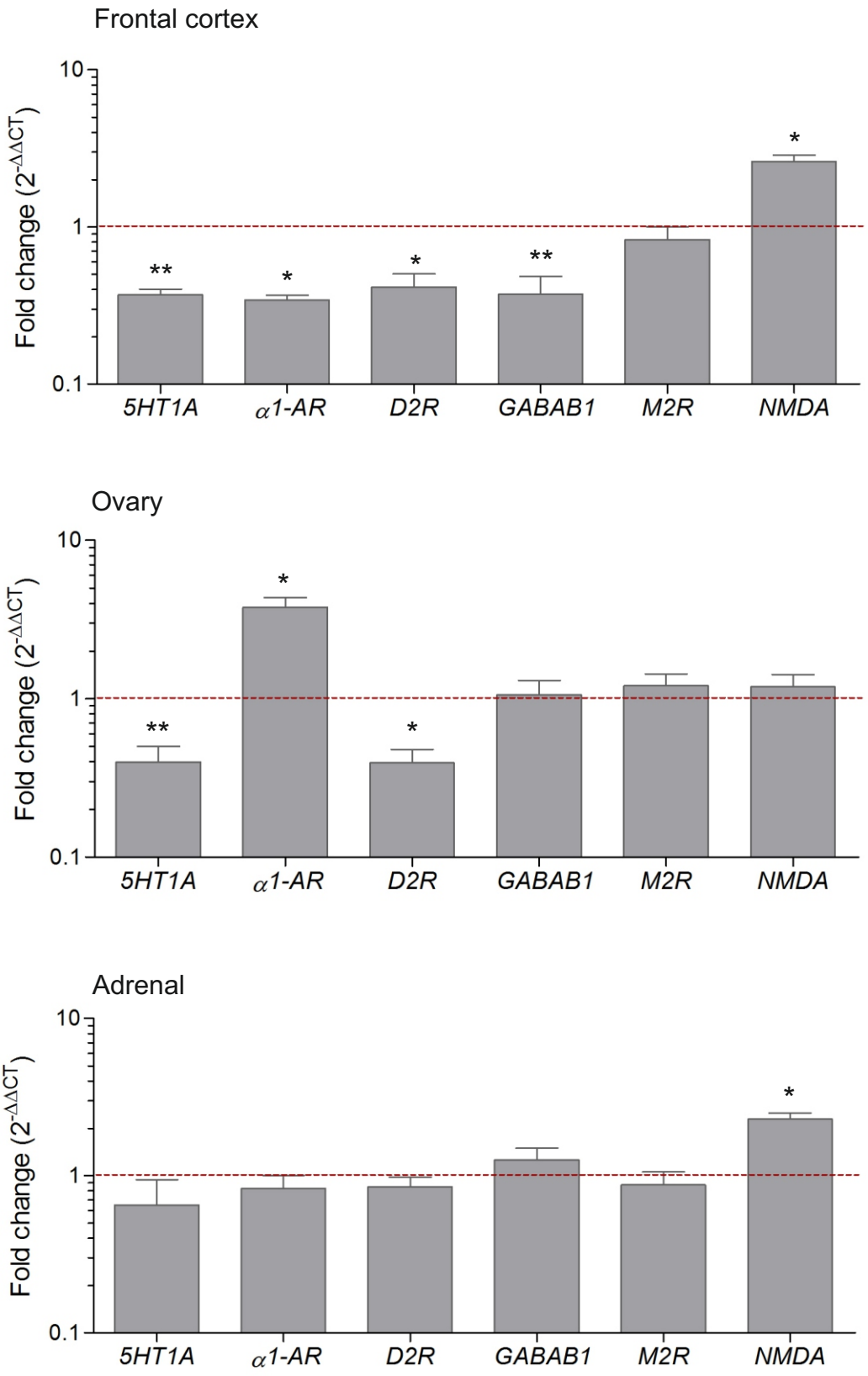

Figure 5.8: Relative gene expression of neurotransmitter receptors. Values are mean fold change in gene expression of PCOS group samples as compared to control samples (represented by red dashed line). ACTH and corticosterone levels in serum. Error bars represent SEM; N=6 per group. *P<0.05; **P<0.01; ***P<0.001 as compared to control group.

DISCUSSION

Reproduction is a fundamental process that requires co-ordination of several organ systems such as hypothalamus, pituitary, hippocampus, ovary and adrenal glands. While reproduction is principally governed by hypothalamic-pituitary-gonadal axis, it is the various neurotransmitters such as serotonin, catecholamines, GABA and glutamate, which directly or indirectly influence this axis. With this reference, the current study was designed to investigate the status of GnRH regulatory neurotransmitter in letrozole-induced PCOS rat model.

Role of neurotransmitters in Hypothalamic-Pituitary-Ovarian axis

The influence of neurotransmitters on each component of the HPO axis has been documented. GnRH is termed as the master molecule released by GnRH neurons that are scattered along the length of hypothalamus. A number of studies using dual-label immunohistochemistry and *in situ* hybridization has shown that several neurotransmitter and neuropeptide receptors are expressed in GnRH neurons (Smith & Jennes, 2001). In addition to GnRH, these neurotransmitters directly regulate LH and FSH release from the pituitary, although the data regarding this is sparse as compared to that for hypothalamic regulation. In various species including rat, pig, primates and humans, the neuronal network in the ovaries has also been documented with presence of norepinephrine, serotonin, acetylcholine, glutamate and GABA (Dominguez & Cruz-Morales, 2012). These intra-ovarian neurotransmitters help in ovarian cell proliferation, follicular maturation and ovulation. In this regard, neurotransmitter evaluation was performed in these areas of PCOS animals. For ease of understanding, the functions of these neurotransmitters will be discussed one at a time.

Serotonin

Serotonin is the major neurotransmitter that participates in almost every body-function. Neurons containing serotonin are localized to raphe nuclei and they project to GnRH neurons in preoptic area as evidenced by neuron-specific trans-synaptic tracing studies (Campbell and Herbison, 2007). The effect of serotonin on GnRH neurons is biphasic in nature which is mediated by stimulation of 5-HT_{1A} and 5-HT_{2A} receptors (Bhattarai et al., 2014). Activation of 5-HT_{2A} receptor increases GnRH neuronal activity via PKC (Protein kinase C) pathway, while activation of 5-HT_{1A} receptor leads to reduced adenylate cyclase activity and/or increased potassium channel opening which in turn suppresses GnRH neuronal firing (De

Vivo and Maayani, 1986; Bhattarai et al., 2014). Similar to GnRH neurons, serotonin can be stimulatory or inhibitory to LH release from anterior pituitary gonadotrophs. With reference to the above understanding, serotonin levels were measured in PCOS rats. Serotonin content was significantly reduced in the hypothalamus and pituitary of PCOS animals as compared to control, which can be well correlated with decreased tryptophan decarboxylase activity (serotonin synthesis) and increased monoamine oxidase (MAO) enzyme activity. Also, the expression of *5HT$_{1A}$* receptor was decreased in both hypothalamus and pituitary of PCOS animals indicating the reduction in the inhibition of GnRH as well as LH pulse in PCOS condition. A study worth mentioning, in support of these results, has demonstrated attenuated GnRH release when hypothalamic GnRH neuronal cell line GT$_{1-7}$ was treated with 5-HT$_{1A}$ receptor agonist (Wada et al., 2006). Also, treatment of 5-HT$_{1A}$ agonist in ovariectomized steroid-treated rats resulted into inhibition of LH surge (Siddiqui et al., 2000).

Serotonin is found in high concentrations in ovarian follicular fluid and its level fluctuates depending upon the stage of ovulatory cycle (Bodis et al., 1993). Also, serotonin stimulates progesterone and estradiol secretion from cultured pre-ovulatory follicles as well as from human granulosa cells (Tanaka et al., 1993). Reduced levels of serotonin and 5HT$_{1A}$ expression in PCOS ovary might result in the decreased estradiol and progesterone content as seen in letrozole-induced PCOS model (described in Chapter 3).

Norepinephrine & Epinephrine

In addition to serotonin, the role of catecholamines is also known in GnRH regulation. The GnRH neurons of the preoptic area and median eminence receive inputs from brain stem adrenergic neurons as observed by retrograde tracing techniques (Wright & Jennes, 1993; Herbison, 1997). Also, immunoreactivity studies showed the presence of α1-adrenergic receptors on both GT-1 cells and GnRH neurons (Lee et al., 1995; Herbison, 1997) indicative of its role in GnRH physiology. Intraventricular infusion of norepinephrine to estradiol-treated ovariectomized rats resulted in increased GnRH mRNA expression within 1 hour of the treatment (He et al., 1993). Also, infusion of prazosin (α1 antagonist) into the preoptic area of estradiol-treated ovariectomized rats reduces GnRH expression (Weesner et al., 1993; Clarke & Pompolo, 2005). Norepinephrine can also affect LH release Propranolol, a β-adrenergic receptor blocker stimulates NE-induced LH release while treatment of α-antagonist blocked the release of preovulatory LH surge (Krieg & Sawyer, 1976; Leung et

al., 1982). Thus, indicating that stimulatory effect of norepinephrine on LH release is mediated by α-adrenergic receptors while β-adrenergic receptor inhibits LH release.

There are very few reports indicating the direct role of epinephrine on hypothalamic GnRH release. In an early study, blockage of the proestrus surge of LH with LY78335, an inhibitor of phenylethanolamine-N-methyltransferase (an enzyme that converts norepinephrine to epinephrine), could only be reversed by administration of epinephrine in cerebral ventricles (Rubinstein & Sawyer, 1970; Plant & Zeleznik, 2014). This study indicates that epinephrine may also exert stimulatory effect on GnRH and LH release. PCOS rats demonstrated low levels of norepinephrine and epinephrine in both hypothalamus as well as in pituitary with reduced expression of α1-adrenergic receptor. Thus, implying reduction of GnRH and LH stimulatory molecules in PCOS condition.

Norepinephrine and epinephrine innervations are also present within the ovarian cortex and medulla and they facilitate ovarian steroid production as well as folliculogenesis (Lara et al., 1993). Norepinephrine treatment results in decreased release of progesterone and increased release of androstenedione and estradiol from the ovary and this action is mediated through α1- and β-adrenergic receptors (Dominguez & Cruz-Morales, 2012). Current observations include significantly elevated levels of norepinephrine and epinephrine in ovary of the PCOS animals with increased α1-adrenergic receptor expression. In line with our results, higher ovarian sympathetic activity was also observed in estradiol valerate-induced PCOS animals (Lara et al., 1993). Also, women with PCOS exhibited altered catecholamine metabolism as evident by urinary examination (Shoupe & Lobo, 1984). This is also evident in our study as activity of neurotransmitter-metabolizing enzymes were elevated in letrozole-induced PCOS model which may have resulted into increased neurotransmitter metabolites.

Dopamine

Dopamine is another catecholamine affecting GnRH release. Although most of the GnRH neurons display tyrosine hydroxylase immunoreactivity, the role of dopaminergic regulation of GnRH is sparsely known. A study using GFP-tagged GnRH neurons revealed both direct hyperpolarizing effects as well as inhibitory presynaptic actions to GnRH neurons (Liu & Herbison, 2013). Dopamine also inhibited the firing and anteroventral paraventricular (AVPV)-evoked GABA/glutamate postsynaptic currents in ∼50% of the GnRH neurons *in vitro* mediated by D1 and D2-like receptors in male and female mice (Liu and Herbison, 2013). This indicates the role of dopamine as a direct suppressor of GnRH release either

through activating D2 dopamine receptor on GnRH neuron or via affecting GABA/glutamate synapses to GnRH neuron (Liu and Herbison, 2013).

The role of dopamine in gonadotropin release is uncertain, yet it is believed to function in cyclic LH release. Dopamine metabolism decreases prior to the LH surge on proestrus and activation of dopamine receptor results into reduced LH pulsatile release (Plant & Zeleznik, 2014). In another study, steroid-treated ovariectomized rats demonstrated increased LH release upon intraventricular injection of dopamine (Vijayan & McCann, 1978). However, similar results were not obtained in other laboratories (Krieg & Sawyer, 1976; Gallo & Drouva, 1979). Although the exact role of dopamine in LH release is not known, it is thought to be inhibitory as seen in case of GnRH. In contrast to brain, dopamine has direct stimulatory effect on the ovarian steroidogenesis. Although very few reports have indicated the role of dopamine in ovary, a study wherein rat ovarian cells were incubated with D1 agonist, showed increased progesterone secretion, while D2 agonist showed no significant effect on progesterone levels (Mori et al., 1994). In PCOS, reduced levels of dopamine in hypothalamus, pituitary and ovary with decreased D2 dopamine receptor expression indicate suppression of GnRH and LH inhibitor in PCOS pathology which culminates into increased GnRH and LH frequency. Supporting our data, many studies suggest the role of reduced dopaminergic tone in increased LH release in PCOS (Kalro et al., 2001; Gomez et al., 2011). Also, this increase in LH release can be attenuated using dopamine receptor blocker metoclopramide. In addition, treatment of bromocriptine, a D2 receptor agonist, can restore normal menstrual cycle and ovulation in PCOS women (Kalro et al., 2001).

GABA

GABA is the major inhibitory neuron of the central nervous system. Electrophysiological studies have revealed that GABA neurons of AVPV, lateral preoptic area and suprachiasmatic nucleus directly project to GnRH neurons (Plant & Zeleznik, 2014). GABA probably inhibits GnRH release through a direct action on GnRH neurones since these neurons express mRNA for $GABA_A$ and $GABA_B$ receptors (Jung et al., 1998; Smith & Jennes, 2001). $GABA_{B1}$ knockout mice of both sexes demonstrated significantly increased GnRH release as well as GnRH pulse frequency (Catalano et al., 2010). In contrast to inhibitory effect of GABA on GnRH, the effect of GABA is biphasic in cultured pituitary cells. Treatment of GABA or muscimol, a $GABA_{A/C}$ receptor agonist, to cultured anterior pituitary cells results into increased secretion of LH through Ca^{2+} release (Virmani et al., 1990). However, when cultured pituitary cells were incubated with baclofen, a $GABA_B$

agonist, GnRH-induced LH release was inhibited while basal LH secretion did not change (Anderson & Mitchell, 1986; Virmani et al., 1990). This suggests that $GABA_{A/C}$ stimulate basal LH secretion whereas $GABA_B$ suppresses GnRH-induced LH release. In PCOS, reduced inhibition of GnRH and LH by GABA through $GABA_B$ receptor may contribute to increased LH response to GnRH pulse. In contrast to this, another study in prenatally androgenised mouse model of PCOS demonstrated increased GABA innervations to GnRH neuron from arcuate nucleus of hypothalamus (Moore et al., 2015). This disparity in the result is likely due to the fact that Moore and group have used arcuate nucleus for the study while we have used whole hypothalamus, which includes many such nuclei. Also, Moore and group (2015) have used prenatally androgenised model of PCOS and *in utero* androgen exposure can lead to epigenetic changes, which could result in developmental alterations in neural circuits. Very few reports have indicated the role of GABA in ovaries. In one study, GABA, superfused on ovarian surface, demonstrated increased ovarian blood flow, elevated estradiol release and reduced progesterone secretion (Erdo et al., 1985). However, we could not find any difference in GABA and its receptor expression in PCOS ovary, which suggests that GABA may not be involved in the ovarian disturbances caused in PCOS. Yet, to decipher any role of GABA in PCOS condition, further studies with other GABA receptor subtypes may need to be done.

Gutamate

In contrast to GABA, Glutamate is the major excitatory neurotransmitter for GnRH release. The role of Glutamate on GnRH neuron activity has been studied extensively using GT1-7 cell lines. GnRH neurons express both ionotropic glutamate receptors AMPA and NMDA and the role of these receptors on GnRH release has been documented (Ottem et al., 2002; Plant & Zeleznik, 2014). Peripheral and central administration of NMDA stimulates GnRH release in a variety of species including the monkey (Plant et al., 1989; Plant & Zeleznik, 2014). NMDA has also been shown to directly stimulate GnRH release from hypothalamic explants and spontaneous pulsatile GnRH release from hypothalamic tissues is suppressed by NMDA receptor blockade (Plant & Zeleznik, 2014). In addition, mRNA and protein expression study has revealed the presence of vesicular glutamate transporter in gonadotrophs of anterior pituitary (Hrabovszky et al., 2006). Also, double-immunohistochemistry study demonstrated presence of NMDA receptor within the LH and FSH releasing gonadotrophs (Bhat et al., 1995). Incubation of kainic acid, a glutamate agonist to gonadotrophs resulted in increased secretion of LH and FSH. This effect was blocked when cells were treated with 2-

amino-5-phosphonovalerate, a competitive NMDAR antagonist (Zanisi et al., 1994) implying a stimulatory role of glutamate in GnRH and gonadotropin release. In PNA-induced PCOS mouse model, no effect of glutamate was observed in GnRH pulsatility (Moore et al., 2015). However, high glutamate levels and NMDA receptor expression in PCOS animals as observed in the current study, suggest the overstimulation of GnRH and LH release. Furthermore, the activities of GAD and GDH were significantly decreased in PCOS rats while that of GABA-T was markedly elevated suggesting that in PCOS condition the flux of reaction is towards the glutamate and not towards GABA. Thus, it is implied that the excitatory signals are increasing while decreased inhibitory current exists in PCOS condition, which might culminate into continuous secretion of GnRH and LH, leading to increased LH/FSH ratio as observed in the letrozole-induced PCOS model. There was no difference observed for glutamate content and its receptor expression in ovary of PCOS animals compared to control group. Although presence of glutamate receptor NMDA has been found in bovine and human ovaries, till date no report has indicated its role in ovarian physiology.

Acetylcholine

Along with all the above-stated neurotransmitters, the role of acetylcholine in GnRH regulation is also emerging. Light and electron microscopic studies have exhibited the projections of cholinergic neurons into hypothalamic GnRH neurons (Turi et al., 2008). In cultured GT1-7 cell line, acetylcholine stimulates GnRH release through activation of nicotinic receptor whilst inhibitory effect of acetylcholine on GnRH activity was mediated by muscarinic receptor activation (Krsmanovic et al., 1998). Also, in GT1-7 cells, acetylcholine treatment activates M2 muscarinic receptor that further reduces forskolin-induced cAMP production followed by suppression of GnRH release (Arai et al., 2017). *In vivo* pharmacological experiments in rats suggested an estrous cycle-dependent stimulation of GnRH release by selective muscarinic antagonists (Koren et al., 1992). The role of acetylcholine in GnRH-induced LH release has also been reviewed recently (Zemková & Stojilkovic, 2017). Treatment of exogenous acetylcholine to cultured anterior pituitary cells from post-pubertal female rats resulted in decreased response of GnRH-induced LH release. Also, this response of acetylcholine was counteracted when cells were incubated with atropine, a non-selective muscarinic receptor antagonist (Zemkova et al., 2013). Acetylcholine has a very short half-life, due to which we could not assess its content. Instead the activity of acetylcholine esterase (AChE), a metabolic enzyme of acetylcholine, was estimated. PCOS rats demonstrated increased AChE activity in the hypothalamus and

pituitary with decreased expression of M2 muscarinic acetylcholine receptor. The increased activity of acetylcholine esterase in PCOS condition indicates reduced bio-availability of acetylcholine. Thus, current results suggest that acetylcholine-mediated inhibition of GnRH and LH secretion is reduced in PCOS condition. In addition, presence of choline-acetyltransferase (ChAT), a biosynthesizing enzyme for acetylcholine has been documented in growing follicles of rodent, monkey and human ovary. The exact role of acetylcholine in ovary is not very well understood but it is believed that it increases intracellular Ca^{2+} levels and helps in FSH-induced cell proliferation (Mayerhofer & Kunz, 2005). In this regard, we could not find any difference in acetylcholine and M2 receptor expression between PCOS and control group, which indicates that acetylcholine may not be involved in ovarian dysfunction of PCOS condition. However, a recent report has demonstrated increased expression of vesicular acetylcholine transporter (VAChT – a marker of acetylcholine presence) in ovarian peripheral cyst of dexamethasone-induced porcine model, suggesting the involvement of ACh in ovarian cyst formation (Kozłowska et al., 2014). Thereby, further study needs to be carried out to investigate the status of other acetylcholine regulatory molecules in letrozole-induced PCOS ovary.

It is clear from literature that GnRH functionality depends on various neurotransmitter inputs. Our data demonstrated an apparent involvement of neurotransmitter alteration in PCOS condition which was evident by decreased levels of all the neurotransmitter measured except glutamate. Furthermore, activity of neurotransmitter synthesizing and metabolizing enzymes suggest that though synthesis of neurotransmitters is somewhat disturbed, it is the degradation which is largely affected in PCOS condition. Additionally, present data clearly demonstrates that GnRH and LH inhibitory neurotransmitters – serotonin, dopamine, GABA and acetylcholine are reduced while glutamate, a major stimulator of GnRH and LH release is increased in the PCOS condition. These neurotransmitter alterations imply that although major stimulatory neurotransmitters are elevated in PCOS, it is the reduction in the inhibitory neurotransmitters in hypothalamic-pituitary centre which result into increased GnRH and LH pulsatility in the PCOS condition.

In addition to HPO regulation, these neurotransmitters also interact with each component of hypothalamic-pituitary-adrenal (HPA) axis and control its activity. Catecholamines norepinephrine and epinephrine are the major signals to exert bidirectional effect on corticotrophin releasing hormone and ACTH release. The stimulatory action of these catecholamines is mediated by α1-adrenergic receptors, whereas inhibitory effect is through

activation of β-adrenergic receptors (Locatelli et al., 2010). Dopamine binds to its receptors D1 and D2 and stimulates HPA axis via increasing the expression as well as the release of CRH and ACTH (Pivonello et al., 2004). Furthermore, serotonin and acetylcholine are known as potent activators of HPA axis. The involvement of both the serotonin-$5HT_{1A}$ and 5-HT_{2A} and nAChR and mAChR-acetylcholine receptors has been proven in mediating these functions (Contesse et al., 2000; Locatelli et al., 2010). Similarly, activation of NMDA and mGlutR results into increase in CRH and ACTH release, suggesting the role of glutamate as a stimulator of HPA axis (Evanson & Herman, 2015). In contrast to all these neurotransmitters, GABA acts as the most potent inhibitor of HPA axis, influencing CRH as well as ACTH secretion (Locatelli et al., 2010). It is clear from the above-cited references that neurotransmitters affect HPA axis. In this line, data from our study has demonstrated no change in hypothalamic CRH as well as pituitary ACTH secretion in letrozole-induced PCOS model (Chapter 4), thereby suggesting that these neurotransmitters may not be involved in affecting HPA axis in letrozole-induced PCOS rat model. Alternatively, it is possible that the cumulative alteration of neurotransmitters seen in our model may nullify the individual effect of each neurotransmitter, resulting into no net change in the CRH and ACTH system.

Contrary to no effect on hypothalamic-pituitary unit, adrenal steroidogenesis is elevated in letrozole-induced PCOS rat model. In this context, several studies have demonstrated involvement of serotonin in adrenal cortisol production. Administration of both $5HT_{1A}$ and $5HT_{2A}$ agonists can increase corticosterone production from rat adrenal gland (Hofmann et al., 2007). In addition, glutamate agonist can also increase adrenal catecholamine and cortisol secretion (Nishikawa et al., 1982; Gonzalez et al., 1998). Dopamine agonist cabergoline, but not bromocriptine, significantly inhibits both baseline and ACTH-stimulated aldosterone secretion (Pivonello et al., 2004), indicating the inhibitory role of dopamine in adrenal steroidogenesis. Thus, implying the stimulatory effect of serotonin and glutamate on adrenal steroidogenesis while dopamine inhibits corticosteroid production. Letrozole-induced PCOS rat demonstrated no change in neurotransmitter receptor expression in adrenal gland except increased NMDA receptor as compared to control group, thereby indicating the role of adrenal NMDA receptor in increased corticosteroid as well as catecholamine secretion in PCOS condition. In line with our findings, the data suggests that activation of NMDA receptor in adrenal gland increases adrenal responsiveness towards ACTH stimulation; however, the exact mechanism is yet to be elucidated (Gill & Pulido, 2005).

Besides the regulation of endocrine axes, these neurotransmitters are also implicated in several psychiatric manifestations of PCOS patients. In this line, different studies have found the association between altered neurotransmitter status and mood disorders. The vast majority of anti-depressants include inhibitors of monoamine oxidase and serotonin reuptake transporters (SSRI), indicating the role of monoamines serotonin, dopamine and norepinephrine in mood regulation. The levels of serotonin and its metabolite 5-hydroxyindole acetic acid are reduced in the CSF of patients with bipolar depression (Hasler, 2010). Decreased 5-HT_{1A} receptor levels have also been found in various brain regions of depressed patients as well as in anxiety disorders as evidenced by PET study (Drevets et al., 2007; Garcia-Garcia et al., 2014). In addition, altered dopamine and norepinephrine status has been linked to depression. Patients with depression demonstrated a notable reduction in norepinephrine levels due to inhibition of key synthetic enzyme tyrosine hydroxylase (Moret & Briely, 2011). Animal models with depletion of dopamine and norepinephrine also exhibited impairment in the behavioral tasks for depression (Darlington et al., 2009). Furthermore, role of glutamate and GABA is emerging in depression and anxiety disorders. Plasma glutamate levels were elevated whereas GABA content was reduced in patients with depression (Sanacora et al., 2012; Brambilla et al., 2003). Administration of ketamine and related NMDA antagonists has been shown to have antidepressant effects in animal models of anxiety and depression, as well as in humans (Maeng & Zarate Jr., 2007). Alterations in acetylcholine signaling have also been shown to lead to symptoms of depression and anxiety wherein overactive or hyper-responsive muscarinic cholinergic system has been documented (Drevets et al., 2008). All these references suggest that alteration in neurotransmitter profile seen in letrozole-induced PCOS model may result into development of depression and anxiety-like symptoms. This has been addressed in the following chapter.

Conclusion

GnRH is the master molecule of reproduction that is influenced by several intrinsic and extrinsic factors such as neurotransmitters and neuropeptides. Results from our study suggest the co-involvement of several neurotransmitter alterations in the pathophysiology of PCOS. When analysed for the neurotransmitters, all those studied were found to be decreased except glutamate. Further, the levels of neurotransmitters correlated well with neurotransmitter metabolizing enzymes activities and their receptor expression. Alterations in GnRH stimulatory as well as inhibitory neurotransmitters in hypothalamic-pituitary unit could be linked to increased GnRH and gonadotropin mRNA expression and may be increasing the

GnRH pulsatility leading to elevated LH/FSH ratio in PCOS. Furthermore, the dysregulated neurotransmitter profile in PCOS could be the reason for low self-esteem, anxiety, frequent mood swings and depression, features closely associated with PCOS pathology in women. This is the first study which explicitly demonstrates that neurotransmitter modulation may act as a key feature in the development of PCOS and pathology with increasing risk of other co-morbidities such as stress and mood.