

## Appendix A14.7

### The Noradrenergic System

Noradrenaline (NE) was originally named sympathine because it was initially observed being released by sympathetic nerve terminals. The chemical later adopted the name noradrenaline after meeting the criteria as a neurotransmitter in the CNS (see Cooper et al., 1996). Alterations in behaviors regulated by the NE system are highly representative of anxiety and affective disorder symptomology observed in patients (Szabo and Blier, 2001). NE is produced from the amino acid precursor L-tyrosine found in neurons in the brain, chromaffin cells, sympathetic nerves, and ganglia. The first step in synthesis of this catecholamine is hydroxylation of the precursor by the enzyme tyrosine hydroxylase (TH) that must be in the presence of  $\text{Fe}^{2+}$ ,  $\text{O}^2$  and a tetrahydropteridine cofactor. This rate-limiting step and enzyme can be blocked by  $\alpha$ -methyl-para-tyrosine, a drug used to halt synthesis. The product formed is, 3,4-dihydroxyphenylalanine (DOPA), which then becomes decarboxylated by decarboxylase in the presence of vitamin B6, to form dopamine (DA). DA is then taken up from the cytoplasm into vesicles and hydroxylated by DA- $\beta$ -hydroxylase in the presence of  $\text{O}^2$  and vitamin C to form NE. The catabolism of NE is performed, in the presence of an aldehyde reductase, by MAO-A. This reaction then yields 3,4-dihydroxyphenolglycol (DOPEG). Once this product is excreted, catechol-O-methyl transferase (COMT) in the presence of S-adenosylmethionine induces the formation of 3-methoxy-4-hydroxy-phenylglycol (MHPG). The dietary depletion of tyrosine and  $\alpha$ -methyl-para-tyrosine has been used in clinical studies to delineate the necessity of NE (and also DA) with respect to anxiety and affective disorders (McCann et al., 1995; Berman et al., 1999; Coupland et al., 2001). MHPG levels have been extensively evaluated in anxiety and affective disorder patients, however, the validity of this measure as representative of CNS function remains unknown.

There are seven NE cell groups in the mammalian CNS, designated as A1-A7. In the brain stem, these are the lateral tegmental neurons (A5 and A7) and the LC (A6; Paxinos et al., 1985). A5 and A7 neurons project to the spinal cord, the brain stem, thalamus, cerebellar and cerebral cortices (Paxinos, 1995). In general, the projections from A5-A7 are more restricted to brainstem areas and do not interfere with that of the A6. It is this latter cell group that will be the most described due to 1) directly being implicated in anxiety and affective disorders and treatment thereof and 2) quite possibly represents the best chemically characterized nucleus in the rat brain. The name *locus coeruleus* was derived from the Greek because of its saddle shape and bluish color (caeruleum). It is located bilaterally in the mammalian brain. The LC is the most widely projecting CNS nucleus known (Foote, 1983), responsible for approximately 90% of the NE innervation of the forebrain and 70% of the total NE in the brain (Paxinos, 1995). For instance, the dorsal hippocampus receives a dense NE innervation exclusively from the LC in the rat (Jones et al., 1977; Menkes

et al., 1983; Sutin and Minneman, 1985). These fibers reach the hippocampus formation by two pathways: the dorsal one travels via the fornix and the cingulum; the ventral one through the ventral and amygdaloid bundle (Loy, 1980; Haring and Davis, 1985).

Under Nissl staining, the LC appears as a densely packed cluster of darkly stained cells in the rostral rhombencephalic tegmentum in which the nucleus is shaped as a tapered cylinder that extends approximately 1 mm in the rostrocaudal axis and 300  $\mu\text{m}$  in diameter at its widest dorsoventral extent (Bezin et al., 1994). The LC traverses along the ventrolateral edge of the IV ventricle. Given that the LC nucleus is fairly homogenous, composed almost exclusively of NE neurons (Paxinos, 1995) with a range of 1400 to 1800 of them. The NE neurons are spontaneous active. This tonic pacemaker activity in LC NE neurons depends on endogenous adenosine 3',5'-cyclic monophosphate (cAMP) levels and involves the cAMP phosphorylation pathway. Endogenous cAMP appears to induce a persistent  $\text{Ca}^{2+}$ -independent/TTX-insensitive inward current that depolarizes the cell membrane (Alreja and Aghajanian, 1995). The specific substrate that may be phosphorylated by endogenous cAMP via protein kinase A to initiate and maintain tonic firing in LC neurons remains to be identified.

### **Noradrenergic Receptors**

The  $\alpha$  and  $\beta$  catecholamine receptors were first discovered more than 50 years ago (Ahlquist, 1948) and later subdivided further into  $\alpha_1$ ,  $\alpha_2$ , and  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$  adrenoreceptors based on pharmacological and functional criteria (Langer, 1974). They are all members of the superfamily of seven transmembrane-domain G protein-coupled receptors. Through experiments using amino acid sequences, signal transduction, and radioligand binding, the adrenoreceptors are now denoted as the  $\alpha_{1A,B,D}$  subtypes being positively coupled to phospholipase C (PLC) and  $A_2$ ,  $\alpha_{2A/D,B,C}$  subtypes which couple negatively to adenylate cyclase, and  $\beta_{1,2,3}$  adrenoreceptors which are all positively coupled to adenylate cyclase (Bylund et al., 1994). The  $\alpha_{2A^-}$ ,  $\alpha_{2B^-}$ , and  $\alpha_{2C^-}$  adrenoreceptors correspond to the human genes  $\alpha_2\text{-C10}$ ,  $\alpha_2\text{-C2}$  and  $\alpha_2\text{-C4}$ , respectively (see Bylund et al., 1994; MacKinnon et al., 1994). The bovine, guinea-pig, rat, and mouse  $\alpha_{2D^-}$  is thought to be a species homologue or variant of the human  $\alpha_{2A^-}$  adrenoreceptor (Bylund et al., 1994), and will be referred to as  $\alpha_{2A/D}$  from hereon.

Adrenoreceptors are located throughout the brain and in the peripheral nervous system. For the purposes of this document, focus on adrenoreceptors located in the raphe and LC brainstem nuclei, hippocampus, and frontal cortex, as the former two nuclei send monoaminergic inputs to the latter forebrain structures. Indeed, these forebrain structures are implicated in the malfunctioning of higher level processing being related to psychiatric disorder symptomology (see Szabo and Blier, 2001). Only receptors that have been implicated in the antidepressant response will be discussed in detail.

## **$\alpha_1$ -adrenoceptors**

Through the use of autoradiography and in situ hybridization techniques, reports have demonstrated that  $\alpha_{1A,B,D}$  receptor subtypes are widely distributed in the rat CNS (Xiong and Sun, 1987). Autoradiographic studies reveal low levels of binding to  $\alpha_1$ -adrenoceptors binding in the hippocampus, moderate level in the raphe nuclei, and a high level in the LC (Unnerstall et al., 1985; Jones et al., 1985; Palacios et al., 1987; Chamba et al., 1991). A high level of mRNA corresponding to  $\alpha_{1A/D}$ - and  $\alpha_{1B}$ -adrenoceptors is present in the hippocampus and raphe nuclei, respectively, whereas only very low levels of  $\alpha_{1A}$ -adrenoceptors were detected in the LC (Pieribone et al., 1994; Nicholas et al., 1996). Given that  $\alpha_1$ -adrenoceptors are mainly not located on NE cells but predominantly found in areas that become innervated by NE neurons, this receptor subtype acts predominantly as a heteroceptor (Nicholas et al., 1996). However, because trace amounts of binding to  $\alpha_{1A}$ -adrenoceptors were present in the LC, this may correspond to heteroceptors located on a few of the non-NE cells in this nucleus. Studies using double labeling with a catecholamine marker would aid in ascertaining this prospectus. Comparing the NE innervation in the rat hippocampus demonstrates that the density of NE containing varicosities is much greater in areas where a lesser amount of  $\alpha_1$ -adrenoceptors is found. This suggests a possible involvement of other adrenoceptors in NE mediated responses in the hippocampus (Zilles et al., 1991).

The  $\alpha_1$ -adrenoceptors act primarily via the phospholipase C-protein kinase C (PLC-PKC) pathway to induce their physiologic actions (Summer and McMartin, 1993). As NE binds to its recognition site on the receptor complex, activation of the 1,4,5 inositol phosphate (PI) pathway is triggered. A cascade effect then results and the subsequent production of diacylglycerol and  $IP_3$  with the release of  $Ca^{2+}$  from internal stores ensue. This release of  $Ca^{2+}$  produces activation of PKC and  $Ca^{2+}$ /calmodulin-dependent protein kinases to mediate phosphorylation/regulation of numerous types of channels and pumps. Also, the release of  $Ca^{2+}$  can directly influence the activation of  $K^+$  channels.

## **$\alpha_2$ -adrenoceptors**

$\alpha_2$ -adrenoceptors are composed of  $\alpha_{2A,B,C,D}$  subtypes, which are located postsynaptically for the most part. Specifically, it has been estimated that approximately 80% of binding sites in the rat brain labeled with the  $\alpha_2$ -adrenoceptor antagonist [ $^3H$ ]idazoxan, is unaltered by the lesioning of NE neurons in the LC with the neurotoxin DSP-4 (Heal et al., 1991). The  $\alpha_{2A}$ - and  $\alpha_{2D}$ -adrenoceptors are orthologous receptors (see Starke, 1987), meaning that these receptors vary only between species, and thus will be referred to as  $\alpha_{2A/D}$ -adrenoceptors from herein. Functionally,  $\alpha_{2A/D}$ -adrenoceptors inhibit adenylate cyclase activity through a  $G_i/G_o$  protein mechanism (Summers and McMartin, 1993), which produce in general an inhibitory action on the cell.

Given the lack of available pharmacologic ligands that discriminate between the different subtypes of  $\alpha_2$ -adrenoceptors, the distribution of these receptors was extensively investigated with autoradiography (Boyajian and Leslie, 1987; Bruning et al., 1987; Hudson et al., 1992; King et al., 1995), immunohistochemical (Aoki et al., 1994; Rosin et al., 1996), and in situ hybridization techniques (McCune et al., 1993; Nicholas et al., 1993; Scheinin et al., 1994; Winzer-Serhan et al., 1997a,b). The  $\alpha_{2A/D}$ -adrenoceptors is proposed to predominate in the rat LC (Wamsley et al., 1992). This is in accord with binding studies in the LC showing a high amount of labeling with the  $\alpha_2$ -adrenoceptor agonist oxytazoline (Albargues et al., 1993). This is also concordant with studies reporting mRNA labeling for the  $\alpha_{2A/D}$ -adrenoceptor in this structure, as well the DR nucleus, CA<sub>3</sub> pyramidal layer of the hippocampus, and cerebral cortex (Nicholas et al., 1993; Scheinin et al., 1994). Furthermore, labeling of  $\alpha_{2C}$ -adrenoceptors with an antibody directed at this subtype is documented in the LC (Rosin et al., 1996). Also observed in the LC were mRNA labeling for  $\alpha_{2C}$ -adrenoceptors, however the  $\alpha_{2B}$ -adrenoceptor subtype was devoid of labeling in the brain except for the thalamus (Nicholas et al., 1993; Scheinin et al., 1994).

With the advancements in molecular biology, utilization of probes have demonstrated that only the  $\alpha_{2C}$ -adrenoceptor subtype can be found at intracellular sites, thus providing another means to be able to discriminate between subtypes of  $\alpha_2$ -adrenoceptors (Rosin et al., 1996). Recently  $\alpha_{2A/D}$ -,  $\alpha_{2B}$ -, and  $\alpha_{2C}$ -adrenoceptor-mutant mice were generated and confirm that  $\alpha_{2A/D}$ -adrenoceptors mainly make up the autoreceptors present on NE neurons with a minor proportion of  $\alpha_{2C}$ -adrenoceptors being demonstrated (Altman et al., 1999). With these genetically altered mice, the conclusion was reached that 5-HT axons which possess  $\alpha_2$ -adrenoceptors are of the same types and in the same proportion found on LC NE neurons as the  $\alpha_{2C}$ - and  $\alpha_{2A/D}$ -adrenoceptor subtypes (Scheibner et al., 2001).

Yet, another drawback to experiments utilizing pharmacological ligands aimed at localizing  $\alpha_2$ -adrenoceptors is the labeling of imidazoline (I) binding sites to which catecholamines do not have affinity. Two classes of I binding sites are defined: I<sub>1</sub> as being labeled with [<sup>3</sup>H]clonidine and [<sup>3</sup>H]para-amino clonidine, of which the parent compound clonidine possess high affinity. Agmatine (decarboxylated arginine) has been proposed to be the endogenous ligand for I<sub>1</sub> (Li et al., 1994). The I<sub>2</sub> receptors are labeled by [<sup>3</sup>H]idazoxan and its non-radioactive form has a high affinity for this receptor subtype (Miralles et al., 1993; Molderings et al., 1993, 1994; Mackinnon et al., 1995). Autoradiographic studies with such compounds suggest the presence of both I<sub>1</sub> and I<sub>2</sub> in the DR and LC (Mackinnon et al., 1995). A recent study from Szabo et al., (1996) rules out the presence of a functional impact of I<sub>1</sub> and I<sub>2</sub> receptors on LC NE neuron activity. This is supported by a study demonstrating that agmatine, which also recognizes  $\alpha_2$ -adrenoceptor binding sites is devoid of pharmacological activity at these receptors (Pinthong et al., 1995) and fails to modify the firing activity of LC NE neurons in vitro (Pineda et al., 1996). Also, the in vivo excitatory effect

of I<sub>1</sub> ligands on the firing activity of LC NE neurons previously described by Pineda et al., (1993) appears to be due to an indirect effect. This has been proposed to be mediated by I receptors located in the medulla and is associated with the paragigantocellularis (PGi) nucleus and modulated by an inhibitory 5-HT mechanism (Ruiz-Ortega et al., 1995; Szabo et al., 1996).

### **$\beta$ -adrenoceptors**

$\beta$ -adrenoceptors are composed of three subtypes denoted as  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$ . All  $\beta$ -adrenoceptors have traditionally been demonstrated to activate adenylate cyclase through a stimulatory G-protein regardless of subtype (Bylund et al., 1994). Additional discussion of the  $\beta_3$ -adrenoceptors will be deferred here, as it is not present in the brain (Nicholas et al., 1996).  $\beta_1$ -receptors have a high affinity for both NE and adrenaline, whereas the  $\beta_2$ -adrenoceptors have preference for adrenaline (Bylund et al., 1994). Both  $\beta_1$ - and  $\beta_2$ -adrenoceptor mRNAs have distinct labeling patterns in the rat CNS and are similar to that observed in primates (Nicholas et al., 1993). In situ hybridization and radioligand binding studies have indicated that  $\beta$ -adrenoceptors do not label cells in the LC or raphe nuclei (Alexander et al., 1975; Sporn and Molinoff, 1976).  $\beta_1$ -adrenoceptors have a much more dispersed labeling in the CNS than  $\beta_2$ -adrenoceptors which displayed the highest labeling in areas of the olfactory bulb, piriform cortex, hippocampal formation, thalamic interlaminar nuclei and cerebellar cortex (Nicholas et al., 1996). Regions of interest which demonstrate high labeling of  $\beta_1$ -adrenoceptors is the ventrolateral pontine and medullary reticular formations (Nicholas et al., 1996), as neurons in these areas exert a potent impact on NE activity in the LC (Aston-Jones et al., 1991).

Electrical stimulation of the LC exerts a brief suppression on CA<sub>3</sub> pyramidal neuron firing in the hippocampus that is followed by a period of excitation (Curet and deMontigny, 1988). It has been pharmacologically elucidated that the inhibition and excitation on CA<sub>3</sub> pyramidal firing from LC stimulation is due to activation of  $\alpha_1$ - and  $\beta$ -adrenoceptors, respectively (Curet and deMontigny, 1988).

Antidepressants downregulate  $\beta$ -adrenoceptors in forebrain structures (Anand and Charney, 2000).

However, this phenomenon occurs with a time-course that often precedes the onset of action of antidepressants as well as occurring with non-antidepressant agents. It is uncertain whether this effect contributes to treatment response (Blier and de Montigny, 1994).

### **Noradrenergic Transporters**

A characteristic feature of neurotransmitters which represent an important function is that upon release in the synaptic cleft, they must have their action rapidly terminated by some process (see Cooper et al., 1996). The NE transporter (NAT) was the first of the monoamine transporters to be cloned in humans, and it transports NE from the synaptic cleft back into the neuron (Pacholczyk et al., 1991). This transporter

was subsequently cloned in bovine (Lingen et al., 1994) and rat brains (Bruss et al., 1997). The NAT is comprised of twelve putative transmembrane domains and bears a large hydrophilic region (extracellular) between region 3 and 4. The rat transporter possesses a 93% and 91% homology of its amino acid sequence in human and bovine, respectively (Bruss et al., 1997; Lingen et al., 1994; Pacholczyk et al., 1991).

Receptor autoradiography with various NE reuptake inhibitors has been used to determine the brain distribution of the NE transporters. Ligands such as [<sup>3</sup>H]desipramine, [<sup>3</sup>H]tomoxetine or [<sup>3</sup>H]mazindol were demonstrated to be of limited use because the first agent listed possesses a high non-specific/heterogenous binding profile and the remaining two bind to other transporters. [<sup>3</sup>H]nisoxetine has proven to be much more of a reliable ligand for this purpose (Tejani-Butt et al., 1990; Gehlert et al., 1995) but is not an antidepressant agent. As one would expect, a high level of NAT is found in the LC, with moderate to high levels found in the dentate gyrus, raphe nuclei, and hippocampus (Tejani-Butt, 1992). This pattern of expression is consistent with the NE innervation to these structures. [<sup>3</sup>H]nisoxetine in the human brain has revealed an even distribution of NAT in the rostral-caudal axis of the LC and is similar to that found in the raphe (Ordway et al., 1997). The NAT is expressed mainly on NE terminals as demonstrated by a drastic reduction of labeling in most post-synaptic regions following NE destruction with the neurotoxin 6-OH-DA or DSP-4 (Tejani-Butt et al., 1990; Tejani-Butt., 1992; Cheetham et al., 1996). This is consistent with in situ hybridization studies showing that mRNA for NAT is detected in the LC of rats (Lorang et al., 1994) and humans (Eymin et al., 1995).

The NAT is dependent on extracellular NE<sup>+</sup> to mediate NE reuptake and the effectiveness of NE reuptake inhibitors in inhibiting NE reuptake (Bruss et al., 1997; Harder and Bonisch, 1985; Friedrich and Bonisch, 1986; Bonisch and Harder, 1986; Lingen et al., 1994). Through a Na<sup>+</sup>/co-transport process, energy for inward solute transfer is coupled to influx of NE down its concentration gradient. Furthermore, the influx of NE is sensitive to intracellular K<sup>+</sup> (Harder and Bonisch, 1985). The uptake of NE is Cl<sup>-</sup> dependent, meaning that the electrogenic process of NE transport is Na<sup>+</sup> and Cl<sup>-</sup> driven (Lingen et al., 1994; Bruss et al., 1997; Harder and Bonish, 1985).

In addition to the electrogenic process, the NAT demonstrates properties of a channel-like pore in that it would transport NE showing an infinite stoichiometry that can be blocked by cocaine and desipramine (Galli et al., 1995; Galli et al., 1996). Given this, as well as the commonality relying on similar ionic events, it was deemed that reuptake inhibitors bind to the same site as the NE recognition site. However, NE was demonstrated to be incapable of displacing [<sup>3</sup>H]nisoxetine from the transporter (Tejani-Butt, 1992), which is in contrast to that of 5-HT for [<sup>3</sup>H]cyanoimipramine (Kovachich et al., 1988). This suggested that all NE reuptake inhibitors may not overlap the binding site for NE. Even though NE is able to inhibit the binding of [<sup>3</sup>H]desipramine, it does so less potently than the reuptake of [<sup>3</sup>H]NE itself (Raisman et al., 1982).

Previous studies based on chimeric proteins have suggested that transmembrane domains 5 to 8 of the NAT are involved in the high affinity binding of TCA drugs, and recent work by Roubert et al., (2001) involving 22 mutants of the human NAT have concluded that domains 6 and 7 may play an important role in the binding of TCA to the NAT, but domain 8 appears likely involved in the high affinity binding of TCA drugs to the NAT. Being that some NE reuptake inhibitors may inhibit the transport of NE by competing for the substrate site, other agents which are not antidepressants but hallucinogens such as ketamine and PCP, as well as some sigma receptors, are able to non-competitively inhibit the reuptake of NE (Baker and Blakely, 1995). Thus, NE may allosterically regulate the binding of NE reuptake inhibitors, as well as reuptake inhibition of NE may modulate the transport of NE by acting non-competitively via a site different from the substrate.

A number of studies suggest that NAT can be regulated by diverse stimuli, neuronal activity, peptide hormones, as well as second messengers being elevated after receptor activation (Baker and Blakely, 1995; Kaye et al., 1997). Recently, cell surface radioligand binding studies demonstrate that activation of mAChR in human (h) blastoma expressing NAT acutely regulates cell-surface density of hNATs. In these cells, mAChR regulation of hNAT involves PKC and direct activation of PKC with phorbol esters influences surface hNAT density (Apparsundaram et al., 1998). Further studies by the group of Blakely using radioligand binding, surface biotinylation, and confocal imaging of immunolabeled transporters strengthen the claim of a major role for change in cell surface distribution as underlying the reductions in NE transport capacity observed after acute PKC activation (Apparsundaram et al., 1998). Recent studies reveal that all monoaminegic transporters (DAT, NAT, and 5HTT) are rapidly regulated by direct or receptor-mediated activation of cellular kinases, particularly PKC (Bauman et al., 2000). PKC activation results in an activity-dependent transporter phosphorylation and sequestration. Protein phosphatase 1/2A (PP<sub>1</sub>/PP<sub>2A</sub>) inhibitors, such as okadaic acid (OA) and calyculin A, also promote monoaminegic transporter phosphorylation and functional downregulation (Bauman et al., 2000). These phenomena that occur beyond the receptor level must be taken into account when considering the ability of antidepressants to alter the reuptake capability of these transporters. In turn, activation of receptors that alter PKC and other second messenger systems may be able to interfere with the transport reuptake process. These interactions should be kept in mind when attempting to develop an antidepressant using a rational approach.

### **NE Metabolites as an Index of Sympathetic Tone**

Plasma NE is derived largely from the vast array of sympathetic nerves innervating blood vessels (Esler, 1982). However, most of the NE released by sympathetic nerves never reaches the circulation but is cleared by a variety of processes, particularly reuptake into presynaptic sympathetic nerve terminals (Kopin,

1985). Intraneuronally, NE is deaminated by monoamine oxidase (MAO) to dihydroxyphenylglycol (DHPG) which diffuses out of the neurons and is O-methylated by catechol-o-methyltransferase to 3-methoxy-4-hydroxy phenylglycol (MHPG). MHPG can be further oxidized (primarily in the liver) to vanillylmandelic acid (VMA).

Ultimately, all NE produced must be excreted (primarily in urine), either as unchanged urine or as one of several metabolites and the sum of NE and its metabolites in urine ( $\Sigma$ NE) should provide a good index of whole-body NE production. This may not be the best index of NE release by sympathetic neurons, however, since some amount of MHPG and VMA derives from intraneuronal NE that was never released but that leaked from storage vesicles into the cytoplasm where it was oxidized by MAO (Goldstein, 1988). Eisenhofer and colleagues (1988) recently estimated that under resting conditions, approximately 64% of DHPG is derived from NE leakage from storage vesicles. In contrast to MHPG and VMA, NMN is formed exclusively by extraneuronal O-methylation from NE that escaped reuptake (Kopin, 1985). In patients with multiple system atrophy, it has been argued that NE and NMN may better reflect NE release by sympathetic neurons, while the deaminated metabolites reflect total NE metabolism (Kopin, 1985). Therefore, the ratio  $(NE+NMN)/\Sigma NE$  is probably a better reflection of actual NE release than is  $\Sigma NE$ . Since the amount of MHPG and VMA excreted per day is some 20 to 30 times greater than the amount of NE and NMN, a small increase in NE release might be reflected in increases in NE and NMN excretion without concomitant increases in MHPG and/or VMA.

Table A14.7a  
PlasmaNE(pg/ml)

Study	Control		UP		BP		Results
	N	Mean $\pm$ SEM	N	Mean $\pm$ SEM	N	Mean $\pm$ SEM	
<b>Supine</b>							
Wyatt et al., 1971	22	200 $\pm$ 17.1	10	360 $\pm$ 25.3			C < U P (p < .01)
Esler et al, 1982	17	225 $\pm$ 8.0	11	427 $\pm$ 49.4			C < UP (p < .05)
Lake et al., 1982 <sup>a,b</sup>	22	270 $\pm$ 6.4	15	530 $\pm$ 33.6	30	420 $\pm$ 10.9	C < <u>BP</u> < <u>UP</u> (p < .05) <sup>d</sup> C < BP (p < .05) <sup>d</sup>
Veith et al., 1983 <sup>c</sup>	8	390.8	14	304.5			UP < C(ns)
Roy et al, 1985 <sup>c</sup>	41	147.2 $\pm$ 6.40	10	219.9 $\pm$ 40.7	7	121.8 $\pm$ 14.7	<u>BP</u> < <u>C</u> < <u>UP</u> (p < .05)

Rudorfer et al., 1985b	12	263.9 ± 33.3	12	233.5 ± 33.8	12	160.7 ± 25.4	<u>BP &lt; UP &lt; C</u>
Siever et al., 1986	21	232 ± 25.3	11	351 ± 81.1 <sup>e</sup>	7	268 ± 29.9	BP < UP (p<.01)
Sevy et al., 1989	14	86.8+/-48.8	14	103+/-35.8			ns
De Villiers et al., 1989 <sup>f</sup>	11	1.42+/-0.6	27	1.36+/-0.46			ns - adolescents
	13	1.05+/-0.46	9	2.48+/-1.45			C<UP p=0.001 - adults
	7	1.52+/-0.59	6	4.0+/-1.58			C<UP p=0.003 - elderly
Rudorfer et al., 1993 <sup>f</sup>	7	1.19+/-0.38	15	1.05+/-0.42			ns
Johnson et al., 1993 <sup>f</sup>			28	2.01+/-0.71 2.73+/-1.42			post imipramine tx 2.52+/-0.57 <sup>g</sup> post fluvoxamine tx 1.94+/-0.90
Veith et al., 1994 <sup>f</sup>	36	0.3+/-0.03	17	0.31+/-0.04 (1 BP)			ns
<i>Yehuda et al.,</i> 1998	13	236.6+/-59.2	12	210.0+/-80.4			ns

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### Standing

Lake et al., 1982	22	500 ± 8.5	8	780 ± 58.6	22	625 ± 13.9	C < BP (p<.05) <sup>d</sup>
Veith et al., 1983 <sup>c</sup>	8	583.7	14	575.2			UP < C (ns)
Roy et al, 1985c	41	290.9 ± 17.4	10	444.9 ± 112.9	7	382.3 ± 69.7	<u>C &lt; BP &lt; UP</u>
Rudorfer et al., 1985b	12	463.6 ± 81.2	12	576.9 ± 69.4	12	438.2 ± 77.8	BP < C < UP (ns)
Rudorfer et al., 1993 <sup>f</sup>	7	2.91+/-0.93	15	2.6+/-1.16			ns

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### Summary:

Supine NE levels of UP patients were significantly higher than controls in **6 of 9** studies; UP patients were significantly higher than BP patients in 3 of 4 studies

Standing NE levels of depressed patients were generally elevated compared with controls; BP patients were consistently lower than UP patients

<sup>a</sup>NE levels (supine and standing) of BP patients tested during manic phase were higher than control levels (p < .05)

<sup>b</sup>Levels approximated from graphed data

<sup>c</sup>Adapted from Rudorfer et al.,1985b

<sup>d</sup>Combined supine and standing measures: C < D (p<.01)

<sup>e</sup>Depressed group variance significantly larger than control group variance (p <.005)

<sup>f</sup>NE in nmol/L

<sup>g</sup> Significant correlation between NE pl and clinical improvement in IMI treated patients (6 weeks)

Table A14.7b

Controlled Baseline Studies of CSF MHPG in Depression and Mania

Study	Patients N			Control Mean %		
	Control	Depressed	Manic	50	100	150
<b>Med-free ≥ 10 days.</b>						
Post et al.,1973	10	25	9	◆ <sup>a</sup>	○ 16.3	
Shaw et ai.,1973	13	22 UP			◆ 10.8	
Berger et al.,1980	23	13			◆ 9.2	
Kosiow et al. 1983	61	99	14		◆ <sup>b</sup> 8.0	○ <sup>b</sup>
Jimerson et al.,1984b	11	20			◆ <sup>a</sup> 8.1	
Widerlov et al.,1988a	10	22			◆ 9.6	
Potter et al.,	48	101			◆	
unpublished data					7.6	
Widerlov et al, 1988	10(51.7+-2.4)	22(51.7+-2.6)			ns	
De Bellis et al 1993c	46(48.0+-9.5)	9(46.7+-14.2) pmol/ml			ns	
Geraciotti et al 1997	10 (137+-24)	10(128+-36) pmol/ml			ns	
<b>Med-free &lt; 10 days</b>						
Wilk et al.,1972	19	5	6		◆ 15.0	○
Shopsin et al., 1974	18	8	13		◆ 15.9	○ <sup>oa</sup>
Subrahmanyam,1975	12	24		◆ <sup>a</sup>	20.6	
Ashcroft et al.,1976	11	7	5		◆ 13.0	○ <sup>ob</sup>
Vestergaard et al.,1978	21	27	4		◆	○

Study	n	n	n	Mean (ng/ml)
Oreland et al., 1981	42	18		10.4
Traskman et al., 1981	45	7		9.1
Gerner et al., 1984	33	34	14	9.7
Asberg et al., 1984	60	26 UP		7.7
		26 BP I		7.7
		6 BP II		9.5

The mean metabolite level (ng/ml) for the control group in each study appears in the 100% column. The shaded I bar indicates standard error (expressed in percentage points) around the control mean expressed as 100%. (Data not available in Shopsin study.)

◆ = Mean for depressed group expressed as % of control mean

o = Mean for manic group expressed as % of control mean

a p < .05 vs. controls

bp < .01 vs. controls

c After fluoxetine treatment MHPG to 4.76 ± 11.6

!  
~r

### Plasma NE Measurements—Additional Caveats

Of the various clinical indices of sympathetic nervous function, the measurements of the plasma concentration of noradrenaline have been used most extensively. Measuring NE (and MHPG) in plasma has the advantages of convenience, accessibility, and control. It allows frequent sampling under a variety of conditions such as controlled physical and mental stress. Indeed, under some conditions, venous plasma NE measures do appear to provide an adequate estimation of average sympathetic outflow. However, plasma NE measurements do have limitations when used as the sole indices of sympathetic activity under a variety of conditions. The plasma NE concentration is determined by the release, reuptake, degradation, redistribution, and partitioning into more than one physiologic space (Linares, 1987). A variety of factors alter the rate at which NE is removed from plasma in humans (Esler, 1982), thereby rendering plasma NE less useful as a valid index of NE release and sympathetic tone. In this context, several investigators (including ourselves) have utilized the rise in plasma NE with upright posture as a clinical index of reflex sympathetic nervous responsiveness. Indeed, a recent study documented parallel increases in sympathetic activity and plasma NE (albeit with several minute time lag), following nitroprusside-induced hypotension in healthy volunteers (Rea et al., 1990). However, plasma NE clearance also falls with upright posture (Esler, 1988), presumably due to reduction in cardiac output and organ blood flows. The relative contribution of these two processes to the observed elevation of Plasma NE is presently unclear but highlights the need to consider alterations in clearance when interpreting changes in plasma catecholamine levels. One approach to overcome the confounding factors of clearance has been to determine NE "spillover" calculated by measuring the ration of

the infusion rate of [<sup>3</sup>H] NE to the specific activity of plasma NE with repeated blood sampling following an infusion of [<sup>3</sup>H] NE to a plateau concentration (Esler, 1982).

An additional concern is that circulating NE, probably consisting for the most part of spillover from the synaptic clefts of sympathetic innervation to arterial vascular walls, reflects peripheral, regionalized sympathetic outflow. Thus, the site of catecholamine sampling is an important factor, and several studies have outlined the limitations of antecubital venous measurements (Goldstein, 1987). Since the forearm is a site of net NE production, antecubital venous NE measurements tend to disproportionately reflect forearm sympathetic nerve activity.

Finally, unlike 24 hour urine collection for 2-3 consecutive days, there is greater intraindividual variability over time, at least for plasma samples at single time points. Given the very short half-life of NE (<10 min) even with repeated plasma measuring, fluctuations may go undetected.

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