5. Patnaik, G K. and Dhawan, B N, *Current Research on Medicinal Plants in India* (ed Dhawan, B N), Indian National Science Academy, New Delhi, 1986, pp 45–71.
6 Patnaik, G K. and Dhawan, B N, *Current Research in Pharmacology in India (1982–1987)* (eds Agarwal, K. K, Seth, P. K and Dhawan, B N), Indian National Science Academy, New Delhi, 1994 (in press)
7 Dandiya, P. C. and Sakina, M. R, *Research and Development of Indigenous Drugs* (eds Dandiya, P. C. and Vohra, S D), Institute of History of Medicine and Medical Research, New Delhi, 1989, pp. 34–44
8 Dhawan, B N, *Proc Indo-US Symposium on Mental Health and Neurosciences in the Decade of the Brain* (eds. Srinivasa Murthy, R and Koslow, S H), National Institute of Mental Health, Rockville, 1994 (in press)
9 Bhattacharya, S K, Bose, R, Dutta, S. C., Roy, A. B and Guha, S R, *Ind J Exp Biol*, 1979, **17**, 598–600.
10 Bhattacharya, S. K., Ray, A. B. and Dutta, S. C., *Planta Medica*, 1975, **27**, 164–170
11. Jamwal, K S, Anand, K. K and Chopra, I C, *Arch Int Pharmacodyn*, 1962, **138**, 400–411
12. Sharma, J D, Dandiya, P C., Baxter, R M and Randell, S. I, *Nature*, 1961, **192**, 1299–1300.
13 Arora, R B, Chatterji, A. K, Gupta, R. D, Arora, C. K and Tandon, P. N, *CNS Drugs* (eds Sidhu, G S, Kacker, I K, Sattur, P B and Thyagarajan, G), Council of Scientific and Industrial Research, New Delhi, 1966, pp 118–132
14 Bhide, N. K. and Aimen, R A, *Nature*, 1959, **183**, 1735–1736
15 Sheth, U. K, Vaz, A, Deliwala, C. V and Bellare, R A, *Arch Int Pharmacodyn.*, 1963, **144**, 34–50
16. Singh, H K. and Dhawan, B N., *J Ethnopharmacol*, 1982, **5**, 205–214.
17 Singh, H. K, Rastogi, R. P., Srimal, R. C. and Dhawan, B N, *Phytotherap Res.*, 1988, **2**, 70–75
18. Singh, H K, Srimal, R C. and Dhawan, B N, *Actes I Colloq Europ Ethnopharmacol*, 1990, pp. 319–321
19. Karanth, K S, Haridas, K K, Gunasundari, S and Guruswami, M N, *Arogya*, 1980, **6**, 137–139
20 Chopra, R N., Chopra, G S and Chopra, I C., *Ind J Med Res*, 1942, **30**, 155–171
21. Chopra, R. N and Chopra, G S, *Ind J. Med Res*, 1935, **23**, 359–389
22 Dhawan, B N, *Structure–Activity Relationships of the Cannabinoids* (eds Rapaka, R S. and Makriyannis, A), National Institute on Drug Abuse, Rockville, 1987, pp 189–203
23. Atal, C K, Siddiqui, M. A, Zutshi, V, Alma, V, Johri, R R, Rao, R G. and Kaur, S, *J Ethnopharmacol*, 1984, **11**, 309–317
24. Ghosh, D., Thejomoorthy, P and Veluchamy, *Ind J Pharmacol.*, 1983, **15**, 331–342
25. Nazimuddin, S K, Viswanathan, S, Kulanthaivel, P, Gopalakrishnan, C and Kameshwaran, L., *Ind J Med Res*, 1980, **71**, 476–479
26 Gujral, M L., Kohli, R P, Bhargava, K P and Saxena, P N, *Ind J Med Res*, 1955, **43**, 89–94
27. Palanicherry, S and Nagarajan, S., *J Ethnopharmacol*, 1990, **29**, 73–78.
28 Srimal, R C. and Dhawan, B N, *J. Pharm Pharmacol*, 1973, **55**, 447–452.
29 Ghosh, D and Anand Kumar, A, *Ind J Pharmacol*, 1983, **13**, 391–402
30 Kulkarni, S K and Varma, A, *Ind. J Exp Biol*, 1992, **30**, 885–888
31 Dhawan, B. N, *The Medicinal Plant Industry* (ed Wijesekera, R. O B), CRC Press, Boca Raton, 1991, pp. 77–84
32 Pradhan, S N, Pandey, K and Badola, R. P, *Br J Anaes.*, 1964, **36**, 306–311
33 Dhawan, B N, *Comp. Rend. Cong Internat Phytotherap*, Nice, 1989, pp 27–35.

Peripheral markers for CNS disease

Prahlad K. Seth* and Devika Nag**

*Developmental Toxicology Division, Industrial Toxicology Research Centre, Lucknow 226 001, India
**Department of Neurology, K. G. Medical College, Lucknow 226 003, India

GROWING evidence of a biochemical basis for the alterations in nervous activity, including behaviour and mood, has led the neuroscientists to investigate the precise mechanisms of neuropsychiatric disorders. Experimental studies involving animals and in some cases human volunteers have helped in understanding the pathogenesis of a variety of neurological and neuropsychiatric disorders and actions of drugs and chemicals. Delineation of the role of dopamine receptors in Parkinson's disease and the observations that two environmental chemicals, viz. MPTP and manganese, could produce Parkinsonism-like syndrome is one such achievement. Similarly, copper and aluminium have been shown to play a role in Wilson's disease and Alzheimer's disease, respectively. These studies have helped in the development of effective therapeutic drugs and better management of the patients.

Advances in cellular and molecular neurobiology have established that neurotransmitters and their receptors and cellular signalling play a central role in the functioning of the brain. Ever since the understanding that neurotransmitters play a key role in the functioning of the brain[1], neuroscientists have attempted to study them under clinical conditions using body fluids and post-mortem brain samples from disease-affected individuals. The latter studies have provided some useful information. However, the reliability of the data using post-mortem samples is uncertain due to difficulties associated with proper isolation and preservation of the tissue; this could significantly affect

the biomolecules. Moreover, the choice of age, sex and stage of disease is not possible. Monitoring of the observed biochemical alterations with the advancement of disease or treatment follow-up in living condition is not possible. Studies on cerebrospinal fluid (CSF) contents for metabolites of neurotransmitters in patients after lumbar puncture often pose ethical considerations and are not preferred. Henkins[2] reported low zinc levels in saliva in the syndrome of idiopathic hypogeusia and other conditions with loss of taste.

Neuroscientists have, therefore, attempted to use peripheral tissues and fluids as a marker for studying the function of CNS under healthy and disease conditions. For example, lacrimal fluid, saliva, sweat, urine, blood and blood components, and cerebrospinal fluid have been used for studying the following conditions:

Saliva:	Henkin's syndrome, low zinc levels, hypogeusia
Lacrimal fluid:	Toxic exposure to heavy metals, autonomic disturbances
Sweat:	Inborn errors of metabolism affecting CNS and autonomic system
Urine:	Porphyric polyneuropathy, multiple myeloma, homocystinuria, metachromatic leucodystrophy
Blood:	HLA tissue typing in genetic disorders, e.g. narcolepsy, alpha fetoproteins
Red blood cells:	Acanthocytosis in dystonia, bone marrow tumour, lead polyneuropathy, basophilic stippling
Serum:	Levels of copper (Wilson's disease), raised serum prolactin level in true tonic clonic epilepsy
Mast cells:	Neurofibromatosis
Lympho-cytes:	Neurodegenerative conditions like movement disorder
Platelets:	Parkinson's disease, affective disorders
Cerebro-spinal fluid:	Myelin basic protein (MBP), multiple sclerosis, (↑) brain tumours, (↑) IgA, IgG, IgS, other CSF proteins like glial fibrillary acidic protein (GFAP) (↑) in syringomyelia, (↑) in spongiform encephalopathy (S-100 is increased in Creutzfeldt–Jacob's disease and Alzheimer's dementia). Enolase (14-3-2 protein) are possible precursors or markers of pathology in CNS. Abnormal pyruvate and lactate in some childhood metabolic CNS disorders presenting with mental retardation.

Urine

Screening of metabolites of neurotransmitters in the urine of psychiatric patients suggests altered neurotransmitter function in certain conditions such as depression and schizophrenia[3]. In patients of depression, reduced levels of serotonin[4] have been observed. Sabelli *et al.*[5] have suggested a decrease in urinary phenyl acetic acid (PAA) as a marker for affective disorder associated with panic attacks. Urinary 3-methoxy-4-hydroxy phenyl-glycol aldehyde (MHFG) level has also been measured in depressed patients together with symptomatology[6]. However, urine analysis in psychiatric conditions offers an indirect method for assessing CNS activity and is limited in applicability. Moreover, conditions related to renal malfunction, presence of metabolites of drugs and chemicals could lead to erroneous data. Therefore, these urine-based estimations have not found much clinical application in studying CNS disorders.

Blood components

Whole blood

Several workers have investigated peripheral neurotransmitter levels in neuropsychiatric diseases in terms of their concentration in whole blood. Most of the earlier reports on transmitter analysis in diseased conditions were based on using whole blood. 5-hydroxytryptamine (5-HT) content has been reported to be significantly lower in the blood of individuals with Down syndrome (Mongolism)[7] and elevated in autistic children[8]. Whole blood catecholamines have also been investigated in juvenile obsessive compulsive disorder[9]. In schizophrenia whole blood 5-HT levels are significantly elevated[10]. Interestingly, a recent report has mentioned the utility of a haemoglobin–acetaldehyde adduct as a new biological marker for alcoholism[11]. Most of the present researchers now prefer isolated blood fractions such as plasma/serum for analysis in comparison with whole blood due to the convenience in storage of samples.

Plasma

There have been a large number of studies on the measurement of various neurotransmitters and associated substances in the plasma of diseased persons. Plasma levels of 5-HT[12], Taurine[13], norepinephrine[14] and dopamine[15] have been reported to be altered in patients with migraine, Spielmeyer–Vogt disease and schizophrenia, respectively. Plasma kynuramine and dexamethasone[16] levels have been suggested as good indices for monitoring depressive patients. Plasma dopamine β-hydroxylase activity has also been studied in psychiatric conditions[17]. Often altered plasma levels are indicative of a 'disease effect' or 'phase effect' of the state, as in the case of elevated plasma tetrahydrobiopterin levels in psychiatric patients[18]

Serum

Certain serum enzymes have been evaluated as possible peripheral markers for CNS disorders. Studies have been conducted on serum and CSF α-1-antichymotrypsin level in patients with Alzheimer-type dementia, Down syndrome, vascular dementia, Parkinson's disease, spinocerebellar degeneration, cerebrovascular disease with dementia and Duchenne muscular dystrophy[19]. A significant elevation of α-1-antichymotrypsin was observed specifically in sera and CSF of Alzheimer-type dementia patients, although there was no correlation between serum levels and the degree of dementia. Enzymes like acetylcholinesterase (GH isoenzyme)[20] and creatine kinase[21] have been studied as markers for Alzheimer-type dementia and major depression, respectively. Low dopamine β-hydroxylase has been suggested as a biological sequela of abuse and neglect in children[22]. In certain nonpsychiatric conditions such as follow-up of brain tumour patients after surgery, serum thymidine kinase has been found to be a useful peripheral marker[23]. Both serum and urinary β-hexosaminidase have been suggested as markers of heavily drinking alcoholics[24].

Lymphocytes

With the demonstration of specific neurotransmitter-binding sites such as adrenergic and dopaminergic in peripheral lymphocytes, their responsiveness in certain disease conditions has been studied. Altered ^{3}H-spiperone binding has been reported in lymphocytes of Parkinson's disease patients[25]. Differences in tritiated spiperone binding between neuroleptic responsive and nonresponsive schizophrenic patients have been observed. Masserini *et al.*[26] investigated lymphocyte subsets in schizophrenia in relation to clinical, neuromorphological and treatment variables. Adrenergic receptors in lymphocytes have been studied in conditions such as congestive heart failure[27] and asthma[28]. A reduction of lymphocyte β-adrenoceptor level in chronic alcoholism followed by rapid reversal after ethanol withdrawal has been reported[29]. Lymphocytic glutamate dehydrogenase activity has been measured in physiological aging and neurological diseases[30]. In their report, heat-stable GDH was significantly elevated in aged people while a decrease in activity was observed in Parkinson's and Alzheimer's disease patients. In addition to lymphocytes, elevated state of leucocyte (WBC) adhesiveness/aggregation has also been suggested as a new independent marker of mental stress[31].

Platelets

Blood platelets have been considered as a peripheral model for the central nervous system for quite some time. Initially, platelets were proposed as a model for presynaptic nerve terminals on account of their ability to accumulate, store and release biogenic amines such as 5-hydroxytryptamine, noradrenaline and dopamine[32-35]. Reports on the presence of neurotransmitter and drug receptor proteins in platelets which are altered in several neurological or psychiatric disorders have further strengthened the proposition of platelets as neuronal model. With the demonstration of several neurotransmitters and their receptor sites and related enzymes, platelets can be considered to have multineurotransmitter sites similar to neurons. Similarities between platelet and brain neurotransmitter proteins have been demonstrated immunologically[36] and by the discovery of neuron-specific proteins such as enolase[37, 38] and synaptophysin[39].

The use of blood platelets as a model system for the synaptic apparatus can, however, be justified only for those parameters where it can be shown that blood platelets and nervous tissue share almost identical characteristics. To date, the knowledge of many biochemical mechanisms underlying platelet physiology is still fragmentary. In fact, the functional role of neurotransmitter–receptor binding sites located on the platelet cytoplasmic membranes, i.e. their coupling to a specific transmembrane signalling mechanism, needs precise biochemical and physiological characterization[40].

Some structural aspects of platelets are analogous to those of neurons. Both possess a limiting membrane, mitochondria and dense-cored vesicles where neurotransmitters are stored. Mitochondria provide energy for cellular functioning and localize monoamine oxidase (MAO)[41] for neurotransmitter catabolism like neurons, and the vesicles or granules are storage sites for serotonin, prepacked for release by excitation–secretion coupling. Platelets and neurons differ in some respects[33], e.g. platelets contain alpha granules which store glycogen while neurons do not, and neurons contain a cell nucleus whereas platelets are anucleated. Perhaps very important is also the fact that platelets are not 'innervated' and do not 'innervate'; thus, they lack synapses. The platelets can be considered structurally as a model for the presynaptic serotonergic nerve terminal but cannot serve as a model for synaptosome since the post-synaptic element of the synapse is lacking. However, other platelet membrane receptors may potentially model either presynaptic or postsynaptic neuronal receptors.

Monoamine systems in platelets

Serotonin

Ever since the presence of serotonin was demonstrated in platelets[42], its biosynthesis in platelets has been a

subject of extensive study. The current belief is that human platelets do not synthesize 5-HT[33], rather the 5-HT destined for platelets is believed to be synthesized in enterochromaffin cells of the gut. New platelets from bone marrow have storage granules and transport mechanisms but no 5-HT. When they pass through the gut circulation the new platelets pick up their 5-HT[43].

Uptake and storage

5-HT accumulation in platelets is against a considerable concentration gradient, reaching as high as 200:1 for platelets compared to plasma[44]. Uptake is both by active and passive processes and occurs mainly at two sites: the cytoplasmic membrane and the intracellular 5-HT storage organelles. Of particular interest are the studies with plasma membrane vesicles of porcine platelets since the transport of 5-HT across the membrane can be examined independently from intracellular events[45, 46]. Active uptake of 5-HT is selective, temperature-sensitive and concentration-dependent, exhibiting structural specificity and ion dependency[47]. Active transport system of platelets is similar to the brain in terms of pharmacological and kinetic parameters[48], although differences may exist[49]. Interestingly, it has been observed that antibodies against blood platelets influence 5-HT uptake and imipramine binding in synaptosomes[36]. Metabolic inhibitors, e.g. cyanide, fluoride, dinitrophenol, monoiodoacetate as well as ouabain, in concentrations which do not cause apparent damage to the platelet structure, decrease the uptake of 5-HT[50]. The serotonin transporter system in platelets has been excellently reviewed[51, 52]. At high concentrations (10^{-5} M) the uptake of 5-HT is by passive diffusion[33].

Blood platelets are able to not only accumulate exogenous amine but also store them in a metabolically stable form in specific storage organelles[47]. The 5-HT storage granules are spherical bodies containing a dense aggregate of osmiophilic material surrounded by a unit membrane. The granular amine transfer mechanism does not appear to be classically 'active' since it lacks dependency upon energy. Monoamines have been shown to interact with ATP by electrostatic forces, thus forming storage complexes[53]. Recently, serotonin organelles of rabbit platelets have been shown to contain synaptophysin[37].

Release

Release of 5-HT by platelets has been shown to occur through several mechanisms. Passive efflux[54], disruption induced by external stimuli such as K^+ and Ca^{2+} concentrations, thrombin and platelet activating factors cause the release of platelet 5-HT by a common mechanism known as platelet release reaction. In both platelets and neurons three main types of release, viz. exocytotic release, reserpine-induced release and phenyl- or indolyl-alkylamines-induced release have been observed. Also, similarity in the mechanism underlying these three types of release in platelets and neurons has been outlined[47] although the stimuli for exocytosis may differ.

Degradation

Monoamine oxidase (MAO), responsible for the oxidative deamination of monoamines, and 5-HIAA, the major metabolite of 5-HT, have been demonstrated in platelets. In human platelets, MAO has been found to be of B type, as the enzyme preferentially catalyses the deamination of amines like β-phenethylamine and benzylamine, and is relatively sensitive to the B-type MAO inhibitor deprenyl[55]. The properties of MAO A and B in platelets are similar to those of the corresponding enzymes in the brain[55]. Human blood platelet monoamine oxidase has been proposed as a biological marker for a variety of psychiatric diseases[41].

Catecholamines

Metabolism

Biosynthesis of catecholamines, dopamine, epinephrine and norepinephrine has not been demonstrated as yet in platelets. However, platelets contain the enzyme phenolsulphotransferase capable of catalysing the sulphoconjugation of a wide variety of phenolic compounds, including dopamine, in a manner similar to the brain tissue[56].

Uptake and storage

Adrenaline[57], noradrenaline[58], metaraminol[59] and dopamine[60] accumulate in blood platelets against a concentration gradient at 37°C. Recently, a highly selective, temperature-dependent uptake system for dopamine comprising both high- and low-affinity components has been demonstrated in human platelets[34] which is similar to brain dopamine uptake[3]. This system is an independent one for dopamine as the other sympathomimetic amines, adrenaline and noradrenaline, have no effect. In addition to this, passive diffusion may also account for dopamine uptake[61]. Platelets have been considered as poor investigative models for dopamine reuptake[62]. Similarities in catecholamine uptake system of adrenergic neurons and platelets have also been suggested[63]. The passive uptake components for adrenaline and noradrenaline are dominant over the energy-dependent, carrier-mediated mechanism. Adrenaline, dopamine and, to a lesser extent, noradrenaline

accumulate in the 5-HT storage granules[64] though the amounts are considerably less compared to 5-HT.

Release

Catecholamines may be released from storage granules along with 5-HT during the platelet release reaction. However, no independent release factors or conditions have been reported for them. Born and Smith[57] demonstrated that following incubation with radiolabelled adrenaline, more than half the accumulated radioactivity is present as adrenaline metabolites. In contrast, both dopamine and noradrenaline are only slowly metabolized following accumulation although dopamine at least is a substrate for the platelet monoamine oxidase.

Platelets accumulate dopaminergic neurotoxin MPTP[65], which is rapidly converted to MPP^+ by platelet MAO B[66]. MPP^+ itself is also actively accumulated by the same energy-dependent carrier mechanism as that utilized for transport of 5-HT and dopamine[65]. Blood platelets have been considered as a very useful cell model for studying *in vitro* and *in vivo* mechanisms leading to cell-specific accumulation of the neurotoxin MPTP and MPP^+ (ref. 40).

Amino acids and other compounds

In addition to biogenic amines, platelets can accumulate other neurotransmitters or neuromodulators such as glutamate, aspartate, glycine, taurine and GABA[67, 68]. It has been reported that 75% of GABA present in the blood is concentrated in platelets, suggesting their ability to accumulate and/or synthesize GABA[69]. Blood platelets also contain a relatively high proportion of free amino acids[70] such as glycine, glutamic acid, taurine and aspartate, GABA transaminase has been detected in the lysate of platelets with properties similar to those found in the brain, liver or kidney[71]. Interestingly, the high-affinity GABA uptake system in platelets seems to resemble glial cells[72]. Further, related amino acids inhibit GABA uptake in platelets, suggesting the possibility of a common transport mechanism[72].

Neurotransmitter binding sites in blood platelets

Serotonergic binding sites[33, 53, 73, 74]

In addition to membrane receptors for serotonin uptake[33] and for tricyclic antidepressants[73], other types of membrane receptors for serotonin have been demonstrated in blood platelets. Such receptors seem to differ from the serotonin receptors responding to substances such as ADP, thrombin and collagen. A great deal of speculation exists about different categories of serotonin receptors in the brain. Some investigators categorize them as presynaptic and postsynaptic serotonin receptors regulating the neuronal firing and synaptic functioning of serotonin neurons. Others categorize serotonin receptors by their ability to be stimulated and/or blocked by specific antagonists or their link to adenylate cyclase or different binding ligands. Three subtypes of serotonin receptors have been characterized in the brain so far[75]. At present it is difficult to compare the various subtypes of brain serotonin receptors with those of platelets. However, among the platelet neurotransmitters, serotonin receptors have been the best studied ones. Initially, serotonin membrane receptors of platelets were studied on the basis of physiological parameters (i.e. shape change, aggregation) rather than on purely molecular pharmacological grounds, i.e. ligand binding[76]. Pletscher and his associates explored extensively a 5-HT receptor on the outer membrane of a platelet which mediates shape change reaction – a transition from the physiological discoid form into a spheroid form[77]. This receptor is distinct from the 5-HT uptake site and is stimulated and blocked by various 5-HT agonists and antagonists as well as by drugs that inhibit platelet shape change[77]. The platelet 5-HT receptors have been considered to be a better model for 5-HT receptors in the spinal cord and reticular formation than for 5-HT receptors in the central nervous system.

An elegant series of experiments by Leysen and coworkers has unveiled the presence of putative 5-HT_2 receptors labelled by ^{3}H-ketanserin on blood platelets[78]. This ligand was found to label cat platelet 5-HT_2 binding sites, demonstrating similar pharmacological characteristics as of rat striatal and frontocortical sites. 5-HT_2 receptors in human platelets have also been identified using the antidepressant drug derivative ^{3}H-tetrahydro-transodone, which binds to human brain. In addition to direct binding studies, Leysen's group has shown functional correlations in both brain and platelets 5-HT receptors. Whereas brain 5-HT_2 binding is correlated with animal behaviour (tryptamine-induced chronic seizures, mescaline and 5-hydroxytryptophan-induced head twitches), platelet 5-HT_2 binding is correlated with 5-HT-induced platelet aggregation. The 5-HT_2 receptors of blood platelets do not appear to activate the adenylate cyclase system. Binding of ^{3}H-lysergic acid diethylamide (LSD) in platelets has been suggested as a model for studies on peripheral and central 5-HT receptors in man[79]. The platelet 5-HT_2 receptor has often been proposed[40, 53] as an effective model for 5-HT_2 receptors in the brain and could serve as a window to CNS disorders linked to 5-HT_2 receptors such as anxiety and depression[33].

Dopaminergic binding sites

In the past twenty years, dopaminergic neurotransmission has received considerable scientific

attention[80]. Involvement of the dopaminergic systems in neurological and psychiatric states has provided impetus for studying dopaminergic transmission in CNS functioning[81]. Receptors for dopamine have also been characterized in peripheral tissues like oesophagus[82], gastrointestinal tract[83], ciliary body[84], liver[85], kidneys[86], lymphocytes[25] and in blood platelets[87]. Earlier studies have shown that ^{3}H-haloperidol labels intact blood platelets (platelet-rich plasma) nonspecifically[88].

Adrenergic binding sites

Physiological and pharmacological studies indicate that actions of catecholamines involve both α- and β-adrenergic receptors in several tissues. Adrenoceptors in the CNS have been subdivided primarily into α and β types, which are further differentiated into α1, α2 and β1 and β2 subtypes[89]. Existence of multiple types of α-adrenergic receptors in different tissues and the presence of some of these in the platelets has been demonstrated[90]. Since classification of α-adrenergic receptors is complex and constantly changing due to rapid advances in this field, it is often difficult to draw similarities between the brain and the platelets. The initial observations that platelet aggregation induced by physiological catecholamines could be possibly mediated by adrenoreceptors led to the discovery and characterization of both α-[91] and β-adrenoreceptors[92] in platelets. The observed response to adrenaline seems to depend on the number of stimulatory α-adrenoreceptors present, as well as on the ratio of these receptors to the inhibitory adrenoreceptors present on platelets. It has been suggested by many investigators that α-adrenoceptors on the human platelets are exclusively of the α2 subtype[93]. When α-adrenergic agonists (such as norepinephrine) .occupy the membrane receptors, adenylate cyclase activity is inhibited, cyclic AMP production is decreased and platelets tend to aggregate[94].

Interestingly, α2-adrenoceptors on human platelets, like those on neurons in the rat brain, appear to become subsensitive after long-term administration of tricyclic antidepressant drugs. Further, chronic administration of tricyclics to endogenously depressed patients was found to reduce the number of ^{3}H-clonidine binding sites on the platelet membranes. Observations such as these highlight the fact that specific binding of radiolabelled α2-adrenoceptor ligands to human platelet membrane could be used to evaluate α2-adrenoceptor function in endogenous depression as well as to assess biochemically the therapeutic responsiveness.

Brain and platelet α2-adrenergic receptors exhibit similar quantitative pharmacologic responses to a number of agonists and antagonists[40]. Furthermore, changes in platelet aggregation and α2-adrenergic receptor sites in depressive disorders have been correlated with changes in central adrenoceptor density[95]. Based on all these similarities in pharmacological and kinetic properties between the brain and platelet adrenoceptor sites, the responsiveness of the blood platelet α2-adrenergic sites in certain psychiatric conditions[96] have been suggested to serve as a satisfactory model for investigating certain characteristics of the brain α2-adrenergic receptors[33].

The presence of β-adrenergic, predominantly of β2 subtype binding sites, has also been shown in human platelets[92]. β1-selective antagonists (atenolol, metoprolol) had no influence on isoprenaline-induced cAMP formation in human platelets. The validity of platelet α-adrenoceptors as a model for brain versus vascular adrenoceptor has been suggested[97].

GABA binding sites

There is only one report[98] stating the existence of GABA binding to human platelets using ^{3}H-muscimol as the ligand. The possible role of GABAergic mechanisms in blood platelets has also been reviewed[72].

Drug binding sites

Binding sites for tricyclic antidepressants, noncyclic antidepressants, benzodiazepines, phencyclidine and other drugs have been demonstrated and used in the study of certain neuropsychiatric disorders[99–102].

National scene

Most of the studies conducted in the country on peripheral markers have been confined to blood platelets. Earlier studies conducted by Prof. K. P. Bhargawa's group (King George's Medical College, Lucknow) demonstrated that the uptake of 5-HT in platelets is diminished in hypertensive patients. In a recent study conducted by this group on hypertensive patients, a marked decrease in 5-HT content and increase in 5-HT efflux, accompanied by increase in plasma 5-HT and 5-HIAA levels has been reported[103]. A significant correlation between rise in diastolic blood pressure and these changes in 5-HT kinetics in platelets in these hypertensive cases was observed. This group is continuing their study on uptake of 5-HT and other neurotransmitters in platelets.

A significant amount of work has been done by Seth and his associates on platelet 5-HT and DA-neurotransmitter receptor types. High-affinity binding of ^{3}H-5-HT has been demonstrated to occur on 5-HT uptake sites[104], as inhibitors of 5-HT uptake, namely amitryptaline, imipramine and nortryptaline, were potent inhibitors of binding rather than 5-HT receptor

antagonists like methysergide and cyproheptadine. Recently, they have also observed specific glutamate binding sites in blood platelets[105, 106]. The presence of both DA-D1 and DA-D2 receptor binding sites in platelets have also been shown by this group[107].

Platelet dopaminergic binding sites have been proposed as possible peripheral markers for CNS dopaminergic alterations in neurotoxic and neurological conditions[108]. Exposure to neurotoxicants such as acrylamide, styrene and methylmethacrylate has been reported to lead to a significant increase in (^{3}H)-spiperone binding in platelet and striatal membranes, whereas methyl mercury chloride exposure results in significant decrease of binding in both tissues[109]. Investigations carried out in neurologic conditions such as mental retardation and hyperkinesia reveal an elevation of ^{3}H-5-HT binding compared to controls. Platelets from schizophrenic patients were found to elicit significant elevation in ^{3}H-spiperone (labelling DA-D2 receptors) binding[110]. Interestingly, these alterations have been found to be analogous to changes reported in corresponding CNS sites in these conditions[111].

Using the DA-D2 binding assay, the authors studied human blood platelet membrane in idiopathic Parkinson's disease (IPD). Two subgroups were evident in the 108 IPD cases studied: one exhibiting an increase and the other a decrease in ^{3}H-spiperone binding. It is of interest to note that the patients exhibiting increased ^{3}H-spiperone binding, responded satisfactorily to L-dopa therapy clinically (assessed on H & Y scale and Webester scale). They also showed a downward trend in the ^{3}H-spiperone binding on follow up. The patients who exhibited a decreased ^{3}H-spiperone binding, showed a poor clinical response as well as no significant change in the binding of ^{3}H-spiperone following L-dopa treatment. A significant decrease in platelet 5-HT_2 receptor was observed in IPD cases. As in the case of 'dopamine receptors' no correlation of clinical staging with the 5-HT_2 receptor alterations was observed. The activity of monoamine oxidase in platelets was found to be increased in Parkinson's patients. A significant decrease in platelet aggregation induced by ADP (32%) and EPN (60%) was also observed in Parkinson's cases.

Conclusion

The measurement of transmitter-related parameters in human peripheral tissues and body fluids not only allows intraspecific analysis under desired clinical supervision but also the data may be obtained relatively painlessly and with little risk to the patient. A major advantage of peripheral markers is that investigative tissue can be obtained at various stages of an episodic disease process and many time-based fluctuations may be monitored in the same individual; hence, excellent control of biological variation is possible. At present, the search for markers in psychiatry has attracted significant attention of both clinicians and neurochemists. In spite of current limitations, biological markers may ultimately increase the precision of clinicians' nosologic and therapeutic decisions. Besides, their use in monitoring of patients during clinical examination is another attraction.

Researchable questions

In spite of certain unanswered questions, platelets continue to be an attractive peripheral model for the central nervous system to biochemists, neurobiologists and clinicians. Their morphological and biochemical similarities with neurons and the ease with which they could be repeatedly obtained from the same human being, combined with the reports that alteration in platelets reflect the changes occurring in the brain in live situations, makes them the most viable peripheral model. Most of the existing information about the biochemical basis of neuropsychiatric disorders has been derived from the studies of postmortem brain samples. Unfortunately, variations such as tissue collection and preservation procedures and the limited choice of age, sex or the actual stage of disease often lead to variable and even nonreproducible data. Although the availability of current techniques like positron emission tomography (PET) and nuclear magnetic resonance (NMR) makes it possible to study the neurotransmitters and their interaction with receptors in the live brain, the technique is expensive and not available easily in all the hospitals and biomedical centres. This further makes blood platelets study an attractive alternative.

The existing literature suggests that platelets could possibly be used to study certain neurological disorders, but there is a need to understand further their characteristics and mechanisms which bring about changes in the platelet receptors under disease conditions like (a) Parkinsonism, (b) schizophrenia, (c) depression, (d) exposure to toxic chemicals and drug exposure, (e) prognosis of disease and (f) drug abuse, especially the effect of cocaine, nicotine, heroin on dopamine and 5-HT receptors or (g) exposure to specific environmental pollutants like cadmium. The uncertainties about the usefulness of the platelet as neuronal model can probably be settled as more data about structure and function of the binding proteins are understood.

1. Bondy, S C., in *Mechanisms of Neurotoxic Substances* (eds Vernadakis, A and Prasad, K. N), Raven Press, New York, 1982, p 25
2 Henkin, R I., Schechter, P J , Hoye, R and Mahern, C F T , *J Am Med Assoc.*, 1971, **217**, 434

3. Dodd, P. R., Hambley, J. W., Cowburn, R. F. and Hardy, J. A , *J. Neurochem* , 1988, **50**, 1333–1345.
4 Curzon, G., in *Central Neurotransmitter Turnover* (eds. Pycock, C. J. and Taberner, P. V.), Croom Helm, London, 1981, pp. 59–80.
5. Sabelli, H C., Javaid, J. I , Fawcett, J., Kravitz, H. M and Wynn, P., *Acta. Psychiatr. Scand* , 1990, **82**(1), 14–16.
6. Carr, V , Edwards, J and Prior, M., *Biol. Psy* , 1988, **23**(6), 560–574.
7. Tu, J., Zellwenger, H , *Lancet* , 1965, **2**, 715–717.
8 Ritvo, E. R., Yuwiler, A and Geller, E., *Arch. Gen. Psychiatr* , 1970, **23**, 566–572.
9. Hanna, G. L., Yuwiler, A. and Cantwell, D. P., *Biol Psy.*, 1991, **29**(8), 738–744.
10. Garelis, E , Gillin, J. C. and Wyatt, R. J., *Am J. Psy.*, 1975, **132**, 184–186.
11. Sillanaukee, P., Seppa, K. and Koivula, T., *Alcohol Alcohol*, 1991, **26**(5/6), 519–525
12. Ribeiro, C , Fontes, A., Cotrim, M. D , Morgadiriho, M T., Ramos, M. I., Santos, E S. and Anastacio De Marcedo, T. D. R., *Cephalagia*, 1990, **10**(5), 213–219.
13 Airaksinen, E. M , *Prog Clin. Biol. Res.*, 1990, **351**, 253–257.
14. Breier, A., Owen, M., Wolkowitz, A., Roy, W , Potter, Z. and Pickar, O , *Am J. Psy.*, 1990, **147**(11), 1467–1470.
15. Sárai, M., Matsunaga, H. and Kimura, S., *Jpn J. Psy. Neurol* , 1990, **44**(3), 557–562
16. Orlikov, A. B , Prakhe, I V. and Ryzhov, I. V., *Zh. Neuropathol. Psikhiatr Im S. S. Korsakova*, 1990, **90**(10), 83–85
17. Markianos, M., Sfagos, C., Bristake, E., *Acta. Pharmacol. Scand* , 1991, **84**(6), 25–28.
18. Hashimoto, R., Ozaki, N., Ohta, T., Kasuhara, Y., Kaneda, N. and Nagatsu, T., *Neuropsychobiol.*, 1991, **23**(3), 140–143.
19 Matsubara, E , Shunsaku, H., Masakuni, A., Mikio, S , Haruyasu, Y., Koichi, O , Kogi, I , Yasua, H. and Katsumi, W., *Ann Neurol.*, 1990, **28**(4), 561–567.
20. Yamamoto, Y., Nakano, S., Kawashima, S , Nakamura, S., Urakami, K., Kato, T and Kameyama, M., *Ann. Clin. Biochem* , 1990, **27**(4), 321–326.
21. Balaita, C., Christodorescu, D., Nastase, R , Iserulescu, C. and Dimian, G., *Rev. Roum Neurol. Psychiatr* , 1990, **28**(2), 127–134.
22. Galvin, M., Shekkar, A , Simon, J., Stitwell, B., Eyck, R. I., Laite, G., Karwisch, G. and Blix, S., *Psy. Res* , 1991, **39**(1), 1–12
23. Amerigo, B., Munari, L , Silvani, A., Solero, C. L. and Bombardieri, E., *Ital. J. Neurol. Sci* , 1990, **11**(4), 359–366
24 Karkkainen, P., *Alcohol. Alcohol*, 1990, **25**(4), 365–370.
25. Lefur, V , Phan, T. and Uzan, A , *Life Sci.*, 1980, **26**, 1139–1148
26 Masserini, C , Vita, A , Basile, R., Morselli, R., Boato, P , Peruzzi, C., Pugnett, L , Ferrante, P. and Cazzullo, C. L., *Schizophr Res.*, 1990, **3**(4), 269–275.
27. Gibelin, P., Sbirrazzuoli, V., Orici, M., Camous, J. P., Lapalus, P. and Morand, P., *Cardiovasc Drug Therap* , 1990, 4(4), 1105–1112
28. Yang, Z. F., *Clin J. Tuberc. Respir. Dis.*, 1989, **12**(3), 143–146.
29 Maki, T , Heikkonen, F., Harkonen, T , Kontulla, K , Harkonen, M and YliRahri, R , *Eur. J Clin. Invest.*, 1990, **20**(3), 313–316
30 Iwatsuji, K , Nakmua, S. and Kameyama, M , *Gerontology*, 1989, **35**(4), 218–224
31 Arber, N , Berliner, S , Tamir, A , Liberman, E., Segal, G , Pinkhas, J and Pironson, M , *Stress Med* , 1991, 7(2), 75–78
32 Stahl, S. M and Meltzer, H Y., *Exp. Neurol* , 1978, **59**, 1–15.
33. Stahl, S. M , in *Physiology and Pharmacology*, Academic Press, New York, 1985, pp. 307–340
34 Dean, B and Copolov, D. L., *Life Sci.*, 1989a, **45**, 401–411.
35. Dean, B. and Copolov, D. L., *Eur. J. Pharmacol.*, 1989b, **173**, 165–169.
36. Trukenmiller, M E., Angel, I , Paul, S. M and Neale, J., *Soc Neurosci. Abstrc.*, 1983, **9**, 563.
37 Marangos, P. J., Campbell, I. C., Suhemechal, D. E., Murphy, D. L. and Goodwin, R. K., *J. Neurochem.*, 1980, **34**(5), 1254–1258.
38. Sternberg, P. E., in *Megakaryocytes Development and Function*: Progress in Clinical and Biological Reseach, 1986, **215**, 373–386.
39. Bhaler, M., Cesura, A. M , Fischer, G., Kuhn, H., Klein, R. L and Da Prada, M., *Eur. J. Biochem.*, 1990, **194**(3), 825–829.
40. Da Prada, M., Cesura, A. M., Launay, J. M. and Richards, J. G., *Experientia*, 1988, **44**(2), 115–126.
41. Youdim, M. B. H., *Experientia*, 1988, **44**(2), 137–141.
42. Bracco, M. and Curti, P. C., *Experientia*, 1954, **10**, 71–72.
43. Erspamer, V., in *Hydroxytryptamine and Related Indole Alkylamines*. Springer-Verlag, New York, Hand Book, *Expt Pharmacol.*, 1966, **19**, 132–181
44. Stacey, R. S., *Br. J Pharmacol* , 1961, **16**, 284–295.
45. Humphreys, C. J., Levin, J. and Rudnick, G., *Mol. Pharmacol.*, 1980, **33**, 657–663.
46. Talvenheimo, J , Fiskes, H., Nelson, P. J. and Rudnick, G., *J. Biol Chem.*, 1983, **258**, 6115–6119.
47. Pletscher, A., *Neurochem Neuropharmacol.*, John Wiley & Sons, New York, 1978, pp. 49–102.
48. Sneddon, J M , *Prog. Neurobiol.*, 1973, **1**(11), 151–198
49. Campbell, J. C. and Todrick, A., *Life Sci.*, 1976, **18**, 1091–1098.
50. Pletscher, A , *Br. J. Pharmacol.*, 1968, **32**, 1–6.
51. Langer, S. Z. and Galzin, *Experientia*, 1988, **44**, 127–130.
52. Marcusson, J. O. and Ross, S. B., *Psychopharmacol* , 1990, **102**, 145–155.
53. Pletscher, A., *Experientia*, 1988, **44**(2), 152–155.
54. Stahl, S M., *Arch. Gen. Psychiatr.*, 1977, **34**, 509–516
55. Edwards, D. J. and Chang, S. S., *Life Sci.*, 1975, **17**, 1127–1134.
56. Toth, L. A. and Elchisak, M. A., *J. Pharmacol. Exp. Therap.*, 1987, **240**, 359–363.
57. Born, G. V. R. and Smith, J. B., *Br. J. Pharmacol.*, 1970, **39**, 765–778
58 McLean, J. R. and Potoczak, D , *Arch. Biochem. Biophys.*, 1969, **132**, 416–422
59. Ahtee, L. and Saarnivaara, L., *J. Pharm. Pharmacol* , 1971, **23**, 495–501.
60. Soloman, M. H., Spirt, N. and Abrams, W. B , *Clin Pharmacol. Therap.*, 1970, **11**, 838–845.
61. Shah, M. S , Burch, E. A., Yates, J. D., May, D. A., Donald, A. G , Freed, J. E. and Pressley, L. C., *Res Comm in Psychol. Psychiatr Behav* , 1980, **5**, 25–35.
62. Malmgrenn, R., *Psychopharmacol.*, 1984, **84**, 480–485.
63 Abrams, W. B. and Soloman, M. N., *Clin Pharmacol. Therap.*, 1969, **10**, 702–712.
64 Da Prada, M. and Picotti, G. B , *Br. J. Pharmacol.*, 1979, **65**, 653–662.
65. Da Prada, M. and Kettler, R., *Clin. Neuropharm* , 1986, **9**, 347–349.
66. Da Prada, M , Richards, J. G. and Kettler, R., *Biomed. Press*, Amsterdam, 1981, pp. 107–145.
67. Mangano, R. M and Schwarcz, R., *J. Neurochem* , 1981, **36**, 1067–1076
68. Mangano, R. M and Schwarcz, R., *J. Neurochem* , 1981, **37**, 1072–1074.
69. Fnna, S J , in *Physico-chemical Methodologies in Psychiatric Research* (eds. Hannin, I and Koslow, S H), Raven Press, New York, 1980, pp 83–101.
70. Gross, R. and Gerok, W , *Thrombos Diathes Haemorh. (Stuttg).*, 1961, **6**,.462–469.
71 White, H L. and Faisan, L. D., *Brain Res Bull* , 1980, **5**, (Suppl), 115–119

72 Oset-Gasque, M J, Launey, J M. and Gonzalez, M P., in *GABAergic Mechanisms in Blood Cells· their Possible Role in GABAergic Mechanism in Mammalian Periphery*, Raven Press, New York, 1986, pp 305–318
73 Langer, S Z and Schoemaker, M, *Neuropsychopharmacol Biol Psy*, 1988, **12**, 193–216
74 Hourani, S M O and Cusack, N J, *Pharmacol Rev*, 1991, **43**(3), 243–298
75 Fozard, J. R, *Trends Pharmacol. Sci*, 1987, **8**(12), 501–506.
76 Pletscher, A and Laubscher, A, *J Neural Transmission*, 1980, **16**, (Suppl), 7–16
77 Pletscher, A, Laubscher, A, Graf, M and Saner, A, *Ann Biol. Clin.*, 1979, **37**(1), 35–39
78 Leysen, J E, Gommeren, W and De Clerk, F, *Arch Int Pharmacodyn. Therap*, 1983, **263**, 322–324
79 Geaney, D P, Schacter, M., Elliott, J. M and Gizahance Smith, D G., *Eur J Pharmacol.*, 1984, **97**, 87–93
80 Strange, P, *Trend. Neurosci.*, 1991, **14**, 43.
81. Creese, I, Morrow, A. L., Hamblin, M. W, Loff, S E. and Sibley, D. R., in *Receptors as Supramolecular Entities* (eds. Biggio, G., Costa, E, Gessa, G L. and Spenno, P F.), Pergamon Press, NY, 1981, pp 1–50
82. Missale, G, *Life Sci.*, 1990, **47**, 447–455
83 Hay, A M, *Gastroentry*, 1977, **72**, 864–869.
84. Lograno, M. D, Daniele, E. and Goroni, S., *Expt Eye Research*, 1990, **51**(4), 495–501
85. McCann, D. J and Tsung Ping Su, *J. Pharmacol Exp Therap.*, 1991, **257**, 547–554.
86. Hietalla, J., Syvalehti, A and Raylta, M., *J. Receptor Research*, 1988, **8**, 753–771
87. De Keyser, J, Dewaele, M, Convents, A, Ebinger, G and Vauquelin, G, *Life Sci.*, 1988, **42**, 1797–1806.
88 Boullin, D. J, Molyneux, D. and Roach, B, *Br. J. Pharmacol*,1978, **63**, 561–566.
89 Hoffman, B. B. and Lefkowitz, R. J, *Ann Rev. Pharmacol Toxicol.*, 1980, **20**, 581–608.
90. Motulsky, H J and Insel, P. A., *N. Engl. J. Med*, 1982, **307**, 18–29.
91. Alexander, R W., Cooper, B and Handlin, R I, *J. Clin. Invest.*,1978, **61**, 1136–1144.
92 Winther, K., Klysner, R., Geisler, A. and Anderson, P. H., *Thromb Research*, 1985, **40**, 757–767.
93 Shattil, S J., Mc Donough, M, Turnbull, J and Insel, P A, *Mol Pharmacol*, 1981, **19**, 179–183
94. Hoffman, B B, Michel, T, Brennemer, T. B and Lefkourtz, R. D, *Endocrinology*, 1982, **110**, 926–932
95 Garcia-Sevilla, J A, Garcia-Vellejo, P and Guiman, J, *Eur J Pharmacol*, 1983, **94**, 359–360.
96 Wirz-Justice, A, *Experientia*, 1988, **44**(2), 145–152
97 Hamilton, C. A and Reid, J L., *Br J Clin Pharmacol*, 1986, **22**, 623–626.
98 Oset-Gasgue, M J and Gonzalez, M P, *Rev Esp Fis*, 1983, **39**(3), 243–248
99 Rehavi, M., Weizman, R and Weizman, A, in *Platelet Membrane Receptors Molecular Biology, Immunology, Biochemistry and Pathology* (eds Jamieson, G A), A R Liss Inc, NY, 1988, vol 100, p 569
100. Plenge, P and Mellerup, E T, *J Neurochem*, 1991, **56**, 248–251.
101. Agarwal, A K, Greco, N J, Ketababa, S, Tandon, N. N, Ricci, K C, Jacobson, A E and Jamieson, G A, *Biochem J*, 1992, in press
102. Bonuccelli, U., Piccini, P, Marazziti, D, Cassano, G B and Muratorio, A, *J. Neural Transm*, 1990, **12**(2), 1939–1947
103 Nityanand, S, Tekwani, B L, Chandra, M, Shankar, K and Singh, B. N, *Life Sci*, 1990, **46**, 367–372
104. Khanna, A S and Seth, P. K., *Biochem. Arch*, 1987, **3**, 31–40.
105. Sah, R, Seth, K and Seth, P K., *Int J. Tox Occup Environ Hlth*, 1991, **1**(1), 185
106. Sah, R, Agrawal, A K and Seth, P K, *Int J Tox Occup Hlth.*, 1991, **1**, 185
107. Khanna, A S., Agrawal, A. K. and Seth, P K, *Biochem Biophys. Res Commun*, 1987, **146**, 983–988
108. Seth, P K. and Khanna, V K, in *Lectures in Neurobiology* (eds Tandon, P N, Bijlani, V. and Wadha, S), Wiley Eastern Ltd, Delhi, India, pp. 60–72
109. Seth, P. K, in *Advances in Toxicology and Environmental Health* (eds Gupta, P K. and Raviprakash, V), Jagmandar Book Agency, Delhi, 1988, pp 341–363
110 Sethi, B B, Kumar, P, Agrawal, A K, Seth, P K and Trivedi, J K., *Acta. Psychiatr Scand*, 1986, **73**, 186–190
111. Seeman, P., in *Dopamine Receptors* (ed. Seeman, P), Alan, R Liss Inc., pp 233–249

Aging – Molecular aspects

Kalluri Subba Rao

Department of Biochemistry, University of Hyderabad, Hyderabad 500 134, India

DUE to the rapid strides of progress in medicine and health care practices, the average human life span has been on the increase during the past 100 years. Consequently, there has been a steady rise in that fraction of the population which is above 60 years. For example, it is estimated that today there are about 60 million persons in India who are above 60 years of age. If the present trend continues, it is expected to touch the 100 million figure soon after the turn of the century. The picture is more alarming in the case of developed countries like USA, if one considers the percentage of population above 60 years.

How old age is viewed in a society is a cultural issue. How an organism becomes old is a biological question. However, deterioration in mental function is both a biological as well as a sociological issue. The study of the aging phenomenon is termed as *gerontology*. If the study emphasizes on the brain or the central nervous