Close encounters of the monoamine kind: immune cells betray their nervous disposition

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doi:10.1111/j.1365-2567.2005.02166.x
Received 4 February 2005; accepted 24 February 2005.
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Summary

Here we review the evidence for immune cells expressing multiple components of the serotonergic and dopaminergic systems that are more commonly associated with the central nervous system (CNS). We discuss where and how peripheral encounters with these biogenic monoamines occur and posit reasons as to why the immune system would wish to deploy these pathways. A full taxonomy of serotonergic and dopaminergic constituents and their workings in component cells of the immune system should facilitate the formulation of novel therapeutic approaches in diseases characterized by immune dysfunction and potentially provide a range of surrogate peripheral markers for registering and monitoring disturbances within the CNS.

Keywords: central nervous system; dopamine; lymphocytes; serotonin; transporters

Immune encounters with serotonin

Location

Serotonin (5-hydroxytryptamine/5-HT), though conventionally considered a neurotransmitter, is produced primarily by the enterochromaffin cells of the gut.[1] A recent study in rhesus macaques revealed that both $CD3^+$ (T cells) and $CD20^+$ (B cells) lymphocytes sit proximal to 5-HT-containing enteroendocrine cells.[2] The 5-HT released at these sites on mechanical (or psychological/emotional) stress is taken up via an active transport mechanism into a number of cell types with platelets providing an especially rich reservoir of serotonin in the circulation. Subsequent release of platelet-stored 5-HT can be rapid, triggered for example by platelet-activating factor, thrombin, complement component fragments C3a and C5a, and immunoglobulin E-containing immune complexes. At sites of inflammation and platelet activation, local concentrations of 5-HT could greatly exceed the relatively low amounts found free in serum. Both primary and secondary lymphoid organs are innervated with nerve fibres,[3] as is skin,[4] while even under non-pathological conditions both $CD20^+$ B cells and activated T-cell 'blasts' can apparently cross the blood–brain barrier.[5] Thus both in the periphery and the central nervous system (CNS) immune cells have the potential to be exposed directly to 5-HT flow (summarized schematically in Fig. 1). Though much play is made of mast cells as an important source of 5-HT in rodents, serotonin is normally absent from human mast cells, being found only when they are associated with discrete pathologies such as in the stroma of carcinoid tumours and in mastocytosis.

Receptors

There are numerous reports of 5-HT modulating immune cell function. Of the 14 currently known receptor subtypes for 5-HT (see Table 1 for nomenclature and molecular characteristics), transcripts for eight have been found in rat immune tissues.[6] Particularly for the promotion of T-cell proliferation, a major target for serotonin action appears to be the 5-HT_{1A} receptor, a property conserved between mammals and fish. B lymphocytes also carry this receptor subtype (among others) and, as with T cells, display NF-κB-dependent up-regulation of both transcripts and protein for the 5-HT_{1A} receptor. At least for rodent splenic B cells, this receptor subtype seems to be involved in the 5-HT-promoted augmentation of mitogenic responses to both lipopolysaccharide and dextran sulphate (reviewed in ref. 6). Interestingly, serotonin, as well as the selective 5-HT_{1A} receptor agonist R-DPAT,

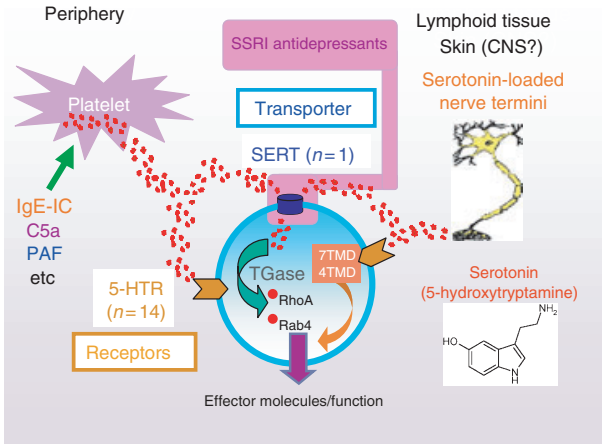

Figure 1. 5-HT stored in platelets or loaded into nerve fibres/cells within the circulation, skin, primary/secondary lymphoid tissues and even possibly the CNS is released on appropriate immune/inflammatory (stress/emotional?) activation. Immune cells exposed to 5-HT flow respond via receptors (up to 14 potentially, majority of which are 7-transmembrane domain (TMD) G-coupled proteins; 5-HT_3 is a 4TMD cation ion channel) and/or the serotonin transporter, serotonin transporter (SERT; also a target for the SSRI antidepressants that block 5-HT uptake). Novel SERT-mediated signalling pathway recently described by Walther and colleagues in platelets (15) may also operate in immune cells where direct SERT-driven change has been shown (11, 13): transported 5-HT via transglutaminase (TGase) used to 'serotonylate' small GTPases such as RhoA and Rab4, both of which impact lymphocyte (and other immune cell) effector function.

were shown recently to increase cell survival and S-phase transition in mouse splenocytes stimulated by T- or B-cell mitogens: these processes being accompanied by translocation of NF-κB to the nucleus.[7] These observations *in toto* point to a potential 5-HT_{1A} receptor/NF-κB-dependent amplification loop in lymphocyte responses to serotonin. Extracellular signal regulated kinase (ERK) phosphorylation is another downstream signalling consequence of ligating 5-HT_{1A} receptors in peripheral blood mononuclear cells.[8] In addition to its activity on lymphocytes, the 5-HT_{1A} receptor has been implicated in up-regulating phagocytic function in mouse peritoneal macrophages.[9]

A recent detailed study highlighted the expression of 5-HT receptors, the signalling pathway they engage, and the biological activity of 5-HT on human dendritic cells (DC). Immature DC preferentially expressed transcripts for the 5-HT_{1B}, 5-HT_{1E} and 5-HT_{2B} receptors, while mature DC mostly expressed 5-HT_4 and 5-HT_7 receptors. The use of isotype-selective receptor agonists showed that 5-HT stimulated 5-HT_3-dependent Ca^{2+} influx in both immature and mature DC whereas 5-HT_1 and 5-HT_2 receptor stimulation induced intracellular Ca^{2+} in immature DC only. Activation of 5-HT_4 and 5-HT_7 induced cAMP elevation in mature DC while enhancing the release of interleukin-1β (IL-1β) and IL-8 and reducing the secretion of IL-12 and tumour necrosis factor-α.[10]

Transporter

Cell lines generated from B lymphocytes transformed with Epstein–Barr virus have provided a rich and productive resource for genotyping polymorphisms in the promoter region and intron 2 of the serotonin transporter (*sert*). Yet a functional role for lymphoid SERT has, until recently, been essentially ignored. We recently reviewed in detail the evidence for lymphocytes expressing functional SERT.[1] This was prompted by our finding of SERT protein and active 5-HT uptake in the constituent cells of Burkitt's lymphoma, a highly aggressive tumour characterized by an extraordinarily high mitotic index and phenotypically resembling B cells found in the germinal centres of secondary lymphoid tissues. The consequence of 5-HT entering these cells was the promotion of apoptosis, a process reversible by transporter blockers [such as the selective serotonin reuptake inhibitor (SSRI) fluoxetine] but not 5-HT receptor antagonists.[11] Interestingly, at higher concentrations, the SSRI are themselves pro-apoptotic for the lymphoma cells, offering the potential for a deliverable therapy in Burkitt's lymphoma.[12] We have since found appreciable levels of SERT protein in a diverse range of B-cell malignancies representing stages of differentiation arrest from pre-B (B-cell precursor acute lymphoblastic leukaemia) through to plasma cells (multiple myeloma). Though 5-HT could affect a number of the malignancies (as registered by cell-cycle arrest), an apoptotic outcome appeared to be specific to Burkitt's lymphoma cells, reflecting their ready propensity to enter this pathway as a result of negligible expression of the Bcl-2 survival protein.[13] SERT may additionally offer a target to amphetamine analogues, such as methylenedioxymethamphetamine (MDMA), in these cells. The impact of MDMA – also known by its street name, 'Ecstasy' – on immune function was recently reviewed by Connor.[14]

Normal B cells also express SERT though in their basal state its levels are low. On stimulation, however, they rapidly acquire readily detectable SERT protein with characteristics similar, if not identical, to the 5-HT transporter found in brain. We are currently undertaking a full molecular comparison of lymphoid and neuronal SERT.[13]

Why should lymphocytes express the uptake machinery for 5-HT? One possible answer could find analogy to the mechanisms and consequences of 5-HT transport in the brain. Thus at local inflammatory sites of high 5-HT production, activated lymphocytes expressing functional SERT could serve to limit/regulate the amount of bioactive indoleamine available for external receptor-triggering; at the same time, any sequestered 5-HT protected from cellular monoaminoxidases (which are undoubtedly present in lymphocytes) could be stored for later release on

Table 1. Molecular, functional and pharmacological information on the human proteins that recognize 5-HT

	5-HT$_{1A}$	**5-HT$_{1B}$**	**5-HT$_{1D}$**	**5-ht$_{1E}$**	**5-ht$_{1F}$**
Structure	7TMD	7TMD	7TMD	7TMD	7TMD
Transduction	$G_{i/o}$	$G_{i/o}$	$G_{i/o}$	$G_{i/o}$	$G_{i/o}$
Selective agonist	8-OHDPAT	none	none	none	LY334370
Selective antagonist	WAY100635	SB236057	BRL15572	none	none
	5-HT$_{2A}$	**5-HT$_{2B}$**	**5-HT$_{2C}$**	**5-HT$_{3}$**	**5-HT$_{4}$**
Structure	7TMD	7TMD	7TMD	4TMD[1]	7TMD
Transduction	$G_{q/11}$	$G_{q/11}$	$G_{q/11}$	Integral cation channel	G_S
Selective agonist	none	none	none	none	BIMU8
Selective antagonist	MDL100907	none	SB242084	Ondansetron	SB204070
	5-ht$_{5A}$	**5-ht$_{5B}$**	**5-ht$_{6}$**	**5-HT$_{7}$**	**5-HTT**
Structure	7TMD	7TMD[2]	7TMD	7TMD	12TMD
Transduction	unknown	unknown	G_S	G_S	Transport
Selective agonist	none	none	none	none	5-HT[3]
Selective antagonist	none	none	SB271046	SB656104	Paroxetine[4]

In accordance with IUPHAR recommendations, receptors in lower case appellation are classified as gene products because of the absence of a defined response in native tissue.

[1]The 5-HT$_3$ receptor is comprised of five proteins which form a ligand-gated cation channel. To date, five different 5-HT$_3$ receptor subunits have been identified (5-HT$_{3A}$, 5-HT$_{3B}$, 5-HT$_{3C}$, 5-HT$_{3D}$ and 5-HT$_{3E}$) although only the 5-HT$_{3A}$ and 5-HT$_{3B}$ have been studied in detail. To date, no pharmacological tools are able to discriminate between the 5-HT binding sites of the expressed isoforms (presence of the 5-HT$_{3A}$ subunit appears essential to form a functional receptor).

[2]Expression of the *5-ht$_{5B}$* gene in humans is doubtful because of the presence of a stop codon in the gene sequence which would result in a heavily truncated, presumably non-functional, protein. A number of additional 'orphan' 5-HT receptors (e.g. 5-HT$_{1P}$) have also been described although no corresponding gene sequences have been identified. TMD, transmembrane domain.

[3]5-HT is the transported substrate of the 5-HTT.

[4]Paroxetine blocks the transporter.

appropriate stimulation at a site distal to the initial exposure. In addition, evidence has been presented for SERT acting not merely as a transport protein but as a signal transducer in its own right when contacting substrate. Moreover, SERT-delivered 5-HT was recently shown to affect signal transduction directly by a novel modification, the so-called 'serotonylation' of small GTPases (see Fig. 1).[15] Thus, SERT appears to be potentially equipped to modify a lymphocyte's functional behaviour in a number of distinct ways. The precise unravelling of these is likely to be an area of intense research, providing fascinating insights into the way serotonin affects immune responses. Moreover, SERT is not restricted to lymphoid cells within the immune system: macrophages for example express both transcripts and protein with the appropriate pharmacology for the transporter.[16]

Immune encounters with dopamine

Location

As for serotonin, there is evidence that the innervation of lymphoid tissues can be dopaminergic in nature, particularly during psychological stress, thus providing a source of this catecholamine for immune cells in an appropriate locale when released on sympathetic activation.[17] However, in addition to this potential paracrine flow is the recognition that immune cells themselves have a capacity for dopamine production whereupon the dopamine could be utilized either in paracrine or autocrine mode. Catecholamines are present in lymphocytes, macrophages and neutrophils and at least in the case of the first two, dopamine is actively synthesized in the cells from tyrosine via the intermediary L-dopa (reviewed in ref. 1).

Receptors

A comprehensive description of dopamine receptor expression in the immune system was undertaken by McKenna and colleagues who used flow cytometry and subtype-specific antibodies to study the distribution among peripheral blood leucocytes.[18] Of the D_1-like receptor family only D_5 was detected, and of the D_2-like receptor family all three receptor subtypes were found. T lymphocytes and monocytes had low expression of

Table 2. Immunomodulatory effects of dopamine

Concentration	Organism/Cell type	Effect	Proposed mechanism	Ref.
Immunosuppressive				
Dop 250 pM	Human T cells ($CD4^+/CD8^+$) activated with IL-2/anti-CD3	~ 50% decrease in thymidine uptake	Activity at D_1-like receptors. Effects reversed by D_1-like receptor antagonist SCH23390	24
Dop 10 nM	Human B and T cells	Apoptosis	Bcl-2/Bax and Fas/FasL alterations	25
Dop 30 nM	Human T cells activated with anti-CD3	↓ thymidine uptake; ↓ cytokine release	D2 and D3 receptors (reversed by receptor antagonism)	26
Dop 100 nM–100 μM	Murine splenic B cells stimulated with anti-μ	Inhibition of proliferation (EC_{50} ~ 10 μM)		27
Dop 10 μM–100 μM	Murine splenic lymphocytes stimulated with Con A/LPS	Dose-dependent inhibition of thymidine uptake		28
Continuous Dop infusion	Murine splenic T cells (*in vivo*)	↓ numbers of IFN-γ-producing cells	D_2 receptor. Effects reversed by D_2R antagonist	29
Dop/Dop agonist 500 nM–1 mM	Human peripheral lymphocytes	Apoptosis	β-adrenergic receptor engagement at least partially responsible for effect	30
6-OHDA 50–250 μM	Human (male) peripheral blood cells	Apoptosis	Oxidative stress (H_2O_2 production). Reversed by antioxidants	31
Dop > 100 μM	Macrophage cell line RAW264.7	↓ proliferation, ↑ apoptosis	Oxidative stress. Effects reversed by ascorbic acid	32
Dop 1 mM	Murine thymocytes	Apoptosis	Oxidative stress. Effects reversed by dithiothreitol	33
L-DOPA 1–100 μM	Human lymphocytes or murine splenocytes stimulated with LPS/ConA	↓ thymidine uptake	Oxidative stress	34
Dop or L-DOPA 10 or 100 μM	Human PBMC stimulated with T-cell (ConA) or B-cell (pokeweed) mitogens	↓ thymidine uptake ↓ IFN-γ release ↓ number of IgG/IgM-secreting cells		35
Dop 10 μM	Murine splenic and thymic cells stimulated with T-cell (ConA) or B-cell (LPS) mitogens	↓ thymidine uptake, EC_{50} ~ 10 μM	Could not be reversed with D_2-like receptor antagonist	36
Dop 100 μM–1 mM	EBV-transformed B-lymphocytes	Impaired mitochondrial function	Suggested to be result of oxidative stress	37
Dopamine (in response to physical stress)	Mouse (*in vivo*)	Impaired Th1/Th2 cytokines and impaired NK response	D_2 receptors (effects reversed with D_2-like receptor antagonists)	38
Immunostimulatory				
Dop < 1 μM	Human T cells	Activation	Signalling via D_2 and D_3 receptors	39
Dop or L-DOPA infusions	Murine splenic T cells (*in vivo*)	Enhanced response of ConA/anti-CD3 stimulated splenocytes	Signalling through D_2 receptors. Effects reversed by D_2 receptor antagonist	29
Dop/Dop-R agonists	Murine splenocytes with ConA or LPS – *in vivo* and *in vitro*	Enhanced proliferation	Receptor-mediated	40
Dop levels ↓ by lesions to dop. neurons	Mouse	Impaired proliferation of splenic lymphocytes; depressed NK activity	Inferred that dopaminergic pathways necessary for immune function	41
Dop receptor agonists	Mouse model (*in vivo*)	Enhanced immune responses		42
100 nM D2/D3 agonist	Human $CD4^+$ or $CD8^+$ T-cells	$CD4^+$ cells shift to TH_1. $CD8^+$ cells triggered to produce IFN-γ	Signalling through D_3 receptor (effects reversed by D_3 antagonist U-maleate)	20

Dop, dopamine; ConA, concanavalin A; IFN-γ, interferon-γ; LPS, lipopolysaccharide; NK, natural killer; PBMC, peripheral blood mononuclear cells; Th1, T helper type 1.

dopamine receptors, whereas neutrophils and eosinophils had moderate expression. B cells and natural killer cells showed higher and more consistent expression. Dopamine receptors D_3 and D_5 were found in most individuals whereas expression of D_2 and D_4 appeared more variable. Dopamine, via a receptor-dependent mechanism, also appears to modify directly the activity of regulatory T cells (T_{reg}).[19] The catecholamine was shown to reduce both the suppressive and trafficking activities of T_{reg} through type 1 receptors (D_1 and D_5) which were abundantly expressed by these regulatory cells. Evidence was presented for dopamine having a negative impact on D_1-like receptor-dependent ERK1/2 phosphorylation in the T_{reg} population.

An intriguing study, recently reported by Ilani and co-workers, indicates the involvement of D_3 receptors in a novel paradigm: the neurotransmitter-mediated brain regulation of peripheral T lymphocytes.[20] Their study appeared to be spurred, at least in part, by the conflictingly low concentrations of dopamine found in the periphery compared to the relatively high amounts seemingly needed to exert discernible effects on immune cells. They resolved this paradox by demonstrating that: (1) only *in-vitro*-activated human T-cell 'blasts' and not resting T lymphocytes respond to D_3 receptor stimulation; (2) peripheral T cells from rats injected with L-DOPA/carbidopa demonstrated properties identical to those of human T-blasts exposed to dopamine; (3) perturbations seen in the model systems were recaptured by peripheral T cells from patients with schizophrenia, postulating here that activated T blasts will have been exposed to increased brain levels of dopamine associated with the disease. The authors propose a 'brain-to-T cells' pathway whereby activated T-blasts that are known to cross the 'blood–brain barrier' are exposed and respond to dopaminergic flow before re-entering the circulation and transmitting knowledge/information of the encounter – via, for example, cytokine release – to peripheral T (and perhaps other immune) cells excluded from the CNS.

Transporter

We recently reviewed the published evidence for lymphocytes expressing a functioning uptake system for dopamine.[1] As for *sert*, lymphocytes offer a readily accessible resource for genotyping polymorphisms in the dopamine transporter (DAT). Correspondingly, Mill and colleagues demonstrated and quantified DAT transcripts in cerebellum, temporal lobe and lymphocytes using quantitative real-time reverse transcription–polymerase chain reaction when investigating the impact on expression of individual variations in the 10-repeat allele of a variable number tandem repeat (VNTR) in the 3′-untranslated region of the transporter.[21] Though also shown to contain immunoreactive DAT protein, no specific function for the transporter in lymphocytes has so far been proffered. Nevertheless, the same arguments offered above as to the teleology of SERT's presence in immune cells could be applied equally to DAT and an exploration of these postulates should prove a fertile area of future research activity.

Overview/synthesis of dopamine's encounters with immune cells

We have attempted in Table 2 to summarize the literature that is available regarding dopamine's impact on immune cells and/or the functioning of an immune response. It is evident that the bulk of reported effects are inhibitory rather than stimulatory. We wish to suggest that this

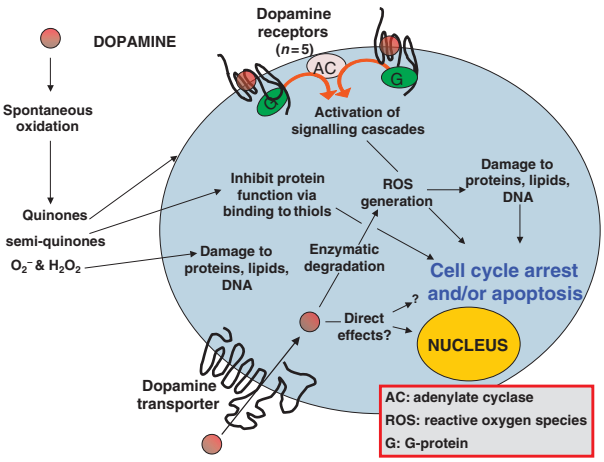

Figure 2. Specific – receptor-mediated: Binding of the monoamine to dopamine receptors alters cAMP levels through modulation of adenylate cyclase (AC) – an enzyme to which these receptors couple (via stimulatory or inhibitory G-proteins). Activation of D1-like and D2-like receptors may inhibit T-lymphocyte activity (26) possibly via increases in cAMP levels (24). *Co*-activation of D1 and D2 dopamine receptors may initiate a unique signalling pathway, not activated by either D1 or D2 receptors alone (43). Alternatively, receptor ligation may initiate a signalling pathway that leads to oxidative stress (44). Specific – transporter-mediated: Dopamine can be actively internalised by cells that express the dopamine transporter (DAT). Intracellular dopamine may bind directly to cellular components such as GTPases to alter their activity (15) or enter the nucleus (45) and bind to nuclear elements (46) to alter transcriptional activity. Cellular dopamine can also chelate iron leading to cell cycle arrest (47). Inside the cell, dopamine can be metabolised by enzymes with the generation of reactive oxygen species (ROS) which may damage cellular components leading to cell-cycle arrest or death. Non-specific mechanisms: Extra-cellular dopamine can undergo auto-oxidation to generate quinones, semi-quinones and hydrogen peroxide (48). Quinones/semi-quinones may bind to thiol groups on cell-surface proteins and inhibit their activity. Hydrogen peroxide generation from dopamine is toxic to immunocytes (31, 37) possibly through the generation of superoxide and hydroxyl radicals which can damage lipids, proteins or DNA, culminating in cell cycle arrest or death.

could reflect in large part the generally accepted 'toxicity' displayed by the catecholamine against a variety of cells, both immune and non-immune. An overview of possible mechanisms that lead to this outcome is presented pictorially in Fig. 2. It needs to be determined which (combinations) of these are operative in specific immune cell responses to dopamine and related compounds.

Closing remarks

The cumulative data for immune cells expressing multiple molecular components that equip them to respond to both serotonin and dopamine are overwhelming. The precise roles of the receptors and transporters for these biogenic monoamines within the immune system are at present, however, less clear. Nonetheless, their eventual disclosure should provide fresh insights and offer new paradigms to the interconnections operating within the neuro–immune axis. Excitingly, this in turn will indicate novel therapeutic targets on immune cells for which pharmacologically well-characterized drugs are already available, a concept recently reviewed by two of us in some detail.[1] Finally, the constituent cells of the circulating immune system provide a readily accessible source, and resource, of material that can potentially be exploited as surrogates to probe for markers of CNS-related disturbances. The examples of *sert* and *dat* polymorphisms have already been cited, the latter in relation to attention deficit disorder for example, the former in a wide variety of psychiatric disturbances including obsessive–compulsive disorder and major depression. Recently, the dopamine D_3 receptor has begun to attract special interest with respect to the 'peripheral marker hypothesis' with mRNA levels reported as being disturbed in individuals with the personality trait of 'persistence', in cigarette smokers, and those suffering from schizophrenia;[22] migraine sufferers show increased D_5 receptor expression.[23] A full taxonomy of the precise complement of serotonergic and dopaminergic components expressed by distinct immune cell subsets, together with the details of their regulation, will greatly facilitate and accelerate both the therapeutic and diagnostic promise being indicated by the intriguing, but limited, studies described so far.

Acknowledgement

We wish to thank the Leukaemia Research Fund for supporting our research in this field.

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