PERSPECTIVES

Methylation reactions at dopaminergic nerve endings, serving as biological off-switches in managing dopaminergic functions

The mechanisms for the regulation of synaptic dopamine (DA) include its release from presynaptic vesicles, its interaction with post-synaptic and pre-synaptic DA receptors, the reuptake of DA, *via* dopamine transporter (DAT), the diffusion of DA and its metabolism by mono-amine oxidase (MAO) and catechol-O-methyl transferase (COMT). DA controls complex and specialized functions including, movements, behavior, mood, perception, reward, and more recently, neurogenesis (Popolo et al., 2004; Reimer et al., 2013) and neuroregeneration (Hoglinger et al., 2004; Yang et al., 2008). These functions are varied and of high fidelity. Movement, as an example, requires regulatory mechanisms for initiating, stopping, slowing-down speeding-up, changing directions, for governing the relentless urges to move in the young and sedentariness in the old as well as in motor-freezing, catalepsy, tremor and stereotypy.

DAT is presented as the key regulator of DA synaptic functions. Its location in the pre-synaptic membrane means that DAT will efficiently transport DA molecule that are closer to the pre-synaptic zone. DA close to the post-synaptic membrane and those coupled to DA receptors will likely escape the reuptake process. For those reasons the reuptake process will reduce extra-synaptic DA, but will not terminate DA neurotransmission. Furthermore, the finding that DAT is located mostly at extra-synaptic sites of dopamine axon terminals, rather than within the synaptic active zone (Deutch and Roth, 1999) raises questions about the efficiency of DAT as the key regulator of dopaminergic synaptic functions. Diffusion will not efficiently remove DA binding to the DA receptors, and DA oxidation occurs mostly in presynaptic endings. So, there are knowledge gaps in matching the regulation of the synaptic activity of DA with the fidelity of the functions that DA modulates. For example, the accepted mechanisms for the regulation of synaptic DA cannot explain why psychotropic dopaminergic drugs take weeks to manifest their actions neither can they explain tolerance and withdrawal related to drugs that modulate DA. Thus, other regulatory mechanisms ought to be involved in the control of the synaptic activity of DA. This perspective will present studies, established findings and a rational hypothesis to show that, between the release process and the second messenger system for DA, biological methylation plays a vital role in the regulation of the synaptic activity of DA that resembles biological off-switches. The operation of these putative switches, as single unit, simultaneously or sequentially, may fit better, the fidelity of the functions that DA controls.

Methylation of dopamine within the synaptic cleft may serve as a phasic down regulating switch for dopamine neurotransmission

The methylation of free DA (f-DA) at the synapse (Axelrod, 1971) irreversibly changes DA to 3-methoxytyramine (3-MT), and 3-MT has been shown to serve as a competing molecule for DA binding (Charlton and Crowell, 2000; Alachkar et al., 2010). Accordingly, the methylation of f-DA in the synapse will reduce the synaptic activity of DA proportionately to the ratio of [3-MT]/[f-DA] and will not terminate the actions of DA in the presence of continuing DA release. So, this methylation function, at DA synapse, resembles a phasic down-regulating switch and may govern measured reduction in locomotor activity.

Direct methylation of DA receptor protein reduces the Vmax and Km for D1 and D2 receptors and may serve as an irreversible and accumulative down-regulating switch

Studies showed that S-adenosyl-L-methionine (SAM) inhibited the binding of ligands to cloned D1 and D2 DA receptors (Lee et al., 2004a). The inhibition was irreversible and both the Vmax and the Km for DA receptor binding were reduced in a concentration dependent manner (Lee et al., 2004a). Moreover, the interactions involved the carboxylmethylation of DA receptors proteins (Lee et al., 2004a), a reaction that is stable at physiological pH, and suggests that methylation permanently down-regulates a fraction of the DA receptors. The process therefore is additive, may serve as a permanent accumulative down-regulating switch for DA synaptic activity, and since methylation reactions are increased with aging, it may explain the progressive reduction of movements that occur during aging, and precipitates the symptoms of Parkinson's disease (PD), of which aging is the major risk factor.

Methylation of membrane phospholipid increases lyso-PTC. Lyso-PTC increases membrane fluidity and reduces the Vmax, but not the Km, for D1 and D2 DA receptors, and may serve as a momentary down-regulating switch for DA synaptic activity

SAM methylates phosphatidyl-ethanolamine (PTE) to phosphatidylcholine (PTC) (Bjornstad and Bremer, 1966; Crews et al., 1980; Lee and Charlton, 2001), and PTC is readily hydrolyzed to lyso-PTC (Lee and Charlton, 2001). Lyso-PTC increases membrane fluidity (Poole et al., 1970) and causes hypokinesia (Lee et al., 2005), and it inhibites the Vmax, but not the Km, for DA D1 and D2 receptors (Lee et al., 2004b). So, lyso-PTC may cause DA receptors to be less available, due to receptor submersion in the soluble membranes. This may explain motor slowing, *e.g.*, malaise, feeling of 'having a bad day' or the 'on-off effects' if the binding is undulating, presenting an on-off switch operation.

Methylation of DA while DA binds to its receptors may serve as a rapid off-switch for DA neurotransmission

It is proposed that DA molecules that are inserted into the DA receptor pocket will be protected from methylation that occurs in the synaptic cleft and catalyzed by soluble COMT. Receptor-bound DA will also escape the reuptake process, but it will be susceptible to methylation, catalyzed by membrane-bound COMT. Methylation of receptor-bound DA will suddenly deprive DA receptors of DA, a phenomenon hypothesized to cause a sudden and complete inactivation of DA synaptic activity, and may serve as a rapid down-regulation switch for DA synaptic functions, and may underlie changes such as catalepsy, motor-freezing or faked-death defensive posture in some animals. The framework for the interaction of DA with its receptor is based on the beta-adrenergic receptor model (Strosberg, 1990), in which DA forms 1 amino and 2 hydroxyls stable 3-points association with its receptors. During methylation, a hydroxyl

of DA is substituted with the methyl of SAM, changing DA to 3-MT, and converting the stable 3-position anchorage to a 2-position unstable anchorage that allows the molecule to uncouple from the receptor. The hydrophobic 3-MT will also interfere with DA molecules entering the receptor pocket, since 3-MT competes with DA for binding sites (Charlton and Crowell, 2000; Alachkar et al., 2010), but 3-MT may be easily displaced since it binds weakly to DA receptors (Alachkar et al., 2010).

Additional supports for the role of methylation in the synaptic activity of DA include: (i) findings that the methyl metabolite, 3-MT, binds DA receptors (Charlton and Crowell, 2000; Alachkar et al., 2010), (ii) manipulations demonstrating that increased brain methylation caused profound DA deficiency states (Crowell et al., 1993; Charlton and Mack, 1994; Charlton and Crowell, 1995), (iii), the utility of 3-MT and homovanillic acid, the oxidized product of 3-MT, as reliable markers for DA synaptic activities and (iv) the association of COMT with dopamine synaptic mechanism (Cooper et al, 1991).

Summary

Methylation of free-DA at the synaptic cleft, methylation of receptor-bound DA, methylation of DA receptors and the action of lyso-PTC on inhibiting DA receptor binding are events that modulate the synaptic activity of DA. They will serve in a coordinated way with DA release and reuptake processes, and diversify the regulation of dopaminergic functions. The release of DA and its binding to its receptors will cause the full activation of DA neurotransmission. The reuptake of DA will cause rapid reduction and will set limits on DA synaptic activity. The methylation of free-DA will cause gradual reduction of DA synaptic activity and the methylation of receptor-bound DA will cause abrupt inhibition of DA synaptic activity. The methylation of DA receptor protein is a stable phenomenon that may serve to progressively down-regulate DA synaptic activity, causing age-related decline in movements. Reduction of the Vmax for DA receptor binding by lyso-PTC may be related to increases in membrane fluidity, facilitating submersion of DA receptors. This perspective ties together long-established findings, recent discoveries and a hypothesis to show that methylation, along with the standard release and uptake processes for DA, may help to explain the fidelity by which the functions that DA controls are regulated. Accepting the role of methylation in the synaptic activity of DA may lead to better ways of managing disorders related to DA synaptic functions. The well-known association of COMT with DA nerve endings may be a further support for the role of methylation in the synaptic activity of DA. Other relevant enzymes that may be strategically associated with DA synapse, including methonine adenosyl transferase (MAT), phenylethanonamine-N-methyl transferases (PENMT) and protein carboxylmethyl transferase (PCMT). The proposed 'off-switches' role of methylation in DA synaptic functions matches findings that excess methylation caused PD-like changes (Crowell et al., 1993; Charlton and Mack, 1994; Charlton and Crowell, 1995). Moreover, the finding that DA depletion impairs precursor cell proliferation in PD (Hoglinger et al., 2004) corresponds with reports that DA promotes neurogenesis (Popolo et al., 2004; Reimer et al., 2013) and neuroregeneration (Hoglinger et al., 2004; Yang et al., 2008; O'Keeffe et al., 2009). The multiple-switch-concept in the regulation of DA synaptic functions is novel, fits well with the biomechanics and redundancy common in biology and requires further investigation.

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