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Review article

Real-time measurements of noradrenaline release in periphery and central nervous system

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Abstract

Noradrenaline (NA) plays important hormonal and neurotransmitter roles in the periphery and central nervous system, respectively. The cells that produce and release NA, namely, adrenal chromaffin cells (ACCs), sympathetic postganglionic neurones and central neurones, show both commonalities as well as profound differences in morphology, physiological function and characteristics of NA secretion.

In order to address disorders which have been associated with the dysregulation of NA release, such as essential hypertension, a better understanding of the molecular mechanisms governing and modulating NA release in neurones is urgently required. Due to profound technical challenges, the molecular basis of NA release has been investigated much more thoroughly in ACCs than in neurones. This review discusses suitable approaches for detecting NA secretion in periphery as well as brain tissues. Membrane capacitance and high-resolution electrochemical measurements have proven particularly useful when combined with fluorescence microscopy. ACCs and peripheral and central NAergic neurones are compared regarding their vesicle morphologies, as well as possible locations of release sites, and the trajectory of secreted NA. Further, current views on the properties of single vesicle release events, including proposed release probabilities in these cell types, are presented.

Keywords: Noradrenaline; Exocytosis; Chromaffin cells; Neurones; Membrane capacitance; Amperometry; Voltammetry; Fluorescence microscopy

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1. Introduction

The catecholamines NA and adrenaline play crucial roles in cardiovascular regulation. The most notable sites of release in the periphery are the chromaffin cells of the adrenal medulla (ACCs) and postganglionic sympathetic

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nerve endings. ACCs are compact neuroendocrine cells stimulated by splanchnic innervation, and by circulating hormones such as angiotensin II, to secrete catecholamines into the bloodstream. Sympathetic neurones display a more prominent polarisation of morphology and function: in the sympathetic ganglia, their cell bodies receive input from preganglionic neurones; they innervate peripheral organs and tissues, releasing NA from varicosities along their axons. Both systems respond to sympathetic activation with catecholamine release, increasing cardiac output and redirecting blood flow by modulating vascular resistance.

In the central nervous system, neurones containing NA (areas A1–6) and adrenaline (C1–3) are located in distinct brainstem nuclei, projecting their axons throughout the CNS (for review, see Moore and Bloom, 1979). Central catecholamines are involved in a vast number of vital brain functions. For example, A2 neurones are implicated in determining the sensitivity of the baroreceptor reflex pathway (Pickel et al., 1977; Talman et al., 1980), and substantial evidence points to a role of the adrenergic C1 neurones in the rostral ventro-lateral medulla in blood pressure control (reviewed in Dampney et al., 2002). It is, further, broadly accepted that the central NA system is involved in regulation of mood, stress responses, alertness and attention, and processing of pain perception (Millan, 2002; Fillenz, 1990a). Numerous clinically used drugs, such as clonidine, methyldopa and monoamine oxidase inhibitors, interact with central catecholaminergic neurones and alter NA release. It is therefore essential to have a clear view of the exact mechanisms which govern NA release from these cells. It appears that most of what is known about NA release comes from studies on peripheral models such as ACCs, using a variety of methods briefly described here. However, these results may only partially apply to central noradrenergic (NAergic) neurones.

The key role of vesicle exocytosis in catecholamine release is well established, although there is evidence for alternative release mechanisms, for example, by inverse uptake. This review compares methods used for measuring catecholamine exocytosis in real time in periphery and brain tissues, and illustrates common features and differences between vesicle characteristics, release sites, and probability and trajectory of release.

2. Measurement of catecholamine release with real-time resolution

2.1. Electrophysiology

Membrane capacitance (C_m) recording has been widely used as an indirect way of measuring catecholamine release from small compact cells like cultured ACCs (Angleson and Betz, 1997; Neher, 1998; Teschemacher and Seward, 2000) or exceptionally large synaptic terminals (Mennerick and Matthews, 1996; Schneggenburger and Neher, 2000). This technique involves voltage clamping of the entire cell or secretory region and computational extraction of C_m change (ΔC_m) information from current responses to voltage stimulation (Gillis, 1995). As C_m is proportional to the total membrane surface area of a clamped cell, it increases upon fusion of exocytotic vesicles with the plasma membrane. The ability to resolve single fusion events by monitoring ΔC_m depends on the vesicle size: Fusion of an ACC dense core vesicle (DCV) with an average diameter of 250–300 nm adds about 2.5 fF to the total cell C_m. In a typical experiment, fusion events in the range of 10–20 large DCVs are easily detected (Teschemacher and Seward, 2000), but signal-to-noise ratio can be increased to the level of single large DCVs (Albillos et al., 1997). The temporal resolution in these experiments is around 10 ms which is compatible with real-time measurement of excitation–secretion coupling (Teschemacher and Seward, 2000; Seward and Nowycky, 1996).

A number of considerations apply when using ΔC_m to deduce catecholamine release: Membrane retrieval by endocytosis counteracts the effect of vesicle fusion and, if occurring with a similar time course, may lead to underestimation of exocytosis. Further, ΔC_m is an indicator for total vesicle fusion over the voltage-clamped region but provides no information on localisation or distribution of release sites (see Schroeder et al., 1994). Finally, the assumption has to be made that vesicles contain a constant concentration of catecholamine, and that full fusion results in total release of the vesicular contents. However, partial fusion events and release from vesicle populations containing different transmitter concentrations lead to variability in the quantal size and therefore distort the relationship between the ΔC_m and transmitter release (see below). Such apparent weakness of the technique can be turned into strength to give additional information when ΔC_m measurement is used in combination with other techniques such as electrochemical or optical approaches (Angleson and Betz, 1997; Zhou et al., 1996). The crucial prerequisite for meaningful C_m measurements is successful space clamp. Therefore, whilst a popular tool for studying exocytosis in small spherical cells such as ACCs, C_m is not an option for measuring catecholamine release from spatially complex structures such as neuronal dendrites and axonal processes.

A more direct electrophysiological approach which can yield information on transmitter release from neurones utilises the activation of specific neurotransmitter receptors on postsynaptic targets. Exocytosis of fast excitatory and inhibitory transmitters such as glutamate, GABA, and ACh which directly activate ion channels can be detected by recording membrane potential responses in a patch-clamped postsynaptic cell or membrane patch (Capogna, 1998; Ninomiya et al., 1997). This technique is widely used in combination with pharmacological block to investigate modulation of release of GABA and glutamate in brain preparations where spontaneous miniature postsynaptic potential frequency often (but not always) correlates well