Editorial

Cardiac microdialysis a powerful tool

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See article by Akiyama et al. [1] (pages 531–538) in this issue.

Tsuyoshi Akiyama and Toji Yamazaki have reported that norepinephrine inhibits acetylcholine release from post-ganglionic cardiac vagal efferents [1]. This is hardly a novel idea since the existence of pre-junctional adrenergic receptors, presumably located on cardiac parasympathetic efferents, has been well documented both physiologically and pharmacologically. What is unique here is that the authors have actually measured steady-state changes in acetylcholine within the interstitium of the working left ventricle. They have done this with an elegant marriage of in situ microdialysis and an ultra-sensitive HPLC-electrochemical detection system.

The report referenced above represents another step forward in the continuing struggle to evaluate the paracrine environment within the myocardium. Much of the early work in this regard was focused on quantifying the dynamics of capillary filtration including the osmotic contribution of interstitial constituents and the measurement of interstitial fluid pressure. Every student of physiology remembers the measurements of interstitial fluid pressure obtained by Guyton from porous spheres inserted into a variety of tissues [2]. Subsequent efforts to explain the regulation of coronary blood flow produced a variety of experimental incursions into the myocardial interstitium. Interstitial constituents were estimated from a variety of sources including micropipettes, electrodes, filter disks, lymph cannulas and pericardial perfusions.

The controversies centered on the adenosine hypothesis were responsible for much of the later drive to investigate the myocardial interstitium. In this regard, Van Wylen et al. [3] reported the application of an existing technique, microdialysis, to estimate interstitial adenosine and its metabolites. Microdialysis, which evolved during the prior decade, had largely been limited to studies evaluating neurotransmitter release within the central nervous system. The active generation of mechanical activity within myocardial muscle provides some theoretical advantages and poses some difficult problems that are not as apparent in stationary tissues. The high metabolic activity and repeated motion of the muscle produces a higher lymph flow and a faster interstitial turnover. The faster equilibration would theoretically allow the observer to make better dynamic measurements. In contrast, the reciprocal muscle activity can break or occlude delicate connections between dialysis windows and their attached inflow and outflow lines. If the sampling device is sufficiently rigid, friction between the probe and the surrounding tissue is a potential source of irritation and inflammation that might obscure the very environment one seeks to sample. Although one can never eliminate tissue damage completely, these dialysis probes are not much more invasive than surgical suture.

The ability to access the extracellular environment in intact tissue makes microdialysis a powerful tool. Cells in situ often behave differently when they are isolated from a native environment which includes nerves, blood vessels and a variety of accessory cell types. In sampling information from the intact tissue, the investigator obtains a better picture of the integrated response. In the process, the investigator necessarily gives up a certain degree of experimental control. The responses of intact and isolated systems can be predictive of one another, however there also can be substantive differences. Surprisingly, investigators using intact systems are often asked to defend why the responses observed differ from those observed in isolated systems (e.g. cultured cells). The differences between the responses in intact and isolated systems can be...
very informative and these differences certainly do not invalidate either approach. However, existing differences clearly emphasize the need to constantly verify the findings obtained in isolated systems by re-evaluating those findings in more complex systems. Microdialysis may serve to provide the observer with a greater degree of control within the intact system.

Microdialysis provides a high degree of spatial discrimination since probes can be placed in very discrete areas. For example, one can easily collect repeated samples from adjacent normoxic and hypoxic regions in the myocardium [7,8]. Dialysis probes have been placed in the rat adrenal medulla [4] and we have been able to reproducibly place probes into the sinoatrial node [5,6]. Surprisingly, in our case both the sympathetic and parasympathetic inputs to the sinoatrial node appear to continue to function largely undisturbed by the insertion of the probe.

Although steady-state measurements are routinely made, the temporal discrimination of dynamic events with microdialysis is significantly more problematic. The ability to separate events that occur in seconds is currently impossible. The extent of this limitation will ultimately be determined by the physics of diffusion but more often, the limitation is practically determined by the sensitivity of the analytical method. In this case, the authors needed to inhibit acetylcholine-esterase, insert two parallel probes and to collect for 20 min to obtain a sample in which they could confidently measure the acetylcholine.

During the last ten years, Akiyama [8–12], Van Wylen [3,7,13–15], Mertes [16,17] and their colleagues have presented a series of technically sophisticated studies that begin to address fundamental questions about the paracrine environment within the heart. The need to validate this approach has required that much of this initial work be conducted to confirm earlier findings collected with other methods. In the current report, Akiyama and Yamazaki elegantly confirm that α-adrenergic receptors participate in the modulation of acetylcholine release. They had nicely demonstrated previously that the recovered acetylcholine most likely originated from post-ganglionic efferent nerve endings. They then added in the more novel finding that the α-receptor process was mediated by modulating calcium conductance through ‘N’-type calcium channels.

Akiyama and Yamazaki aptly demonstrate the potential power of this technique. One can deliver minute concentrations of pharmacological agents into discrete areas of the myocardium and sample the biological response from that same area at the same time. This can often be accomplished without altering systemic hemodynamics or neural activity. As this technique evolves and more investigators begin to explore its enormous potential, we would suggest that where practical, functional controls should be included and physiological limits should be acknowledged. For instance, when the local acetylcholine was reduced, was the local myocardial response consistent with the reduced neurotransmitter release? What is the functional significance of evaluating vagal stimulation at 20 Hz? Although it is time consuming and expensive, it is important to evaluate more than one agent or more than one dose of an agent. This is particularly relevant when negative results are obtained. In this current report on calcium channel antagonists, the N-channel antagonist was effective but the L-channel antagonist, nifedipine, was ineffective. Since size is not the only determinant of permeation through the dialysis membrane, what evidence was presented that nifedipine was able to cross the dialysis membrane or that the dose of nifedipine was adequate?

Since the tissue environment around the dialysis probe is an important determinant of the rate of permeation in either direction, permeation tests in vitro, though very helpful, do not necessarily accurately reflect conditions in vivo. The drug concentrations included in the perfusate are often very high. The higher concentrations are sometimes based on previous experience with related compounds and assume that the permeation rate is very low. Under these circumstances, highly permeant agents could produce extraordinarily high local concentrations that might then compromise their selectivity. In the current study, phentolamine (10^{-7} M) alone was employed to establish the α-adrenergic character of the response. Since all pharmacological selectivity erodes at high concentrations, the proof of α-receptor participation would have been stronger if phentolamine had been shown effective at lower doses or if the phentolamine effect was reproduced by other α-antagonists. Additional support for the α-receptor participation could have been obtained if the norepinephrine effect were duplicated by other α-agonists and was not influenced by either β-agonists or β-antagonists. Finally, the logical assumption was made that the α-receptor was located pre-junctionally on the vagal efferent nerve ending though no morphological evidence was presented. One might argue that the receptor might also have been located on an intrinsic cardiac interneuron or some accessory cell that then served to inhibit acetylcholine release.

Cardiac microdialysis now affords us a unique opportunity to peer into the cardiac interstitium and the paracrine environment of the cardiomyocyte. In addition to the largely methodological considerations cited above, the cellular heterogeneity [18,19] within the myocardial environment suggests that we should begin to acknowledge that the simple neuroeffector construct that proceeds pre-ganglionic nerves to post-ganglionic nerves to myocytes is likely to be far more complex than often appreciated. Finally, though the concerns raised above are by no means comprehensive, we hope they will generate a productive discussion of merits, limitations and opportunities presented by this powerful tool.

References


