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SYMPOSIUM REVIEW

Preclinical and clinical evaluation of autonomic function in humans

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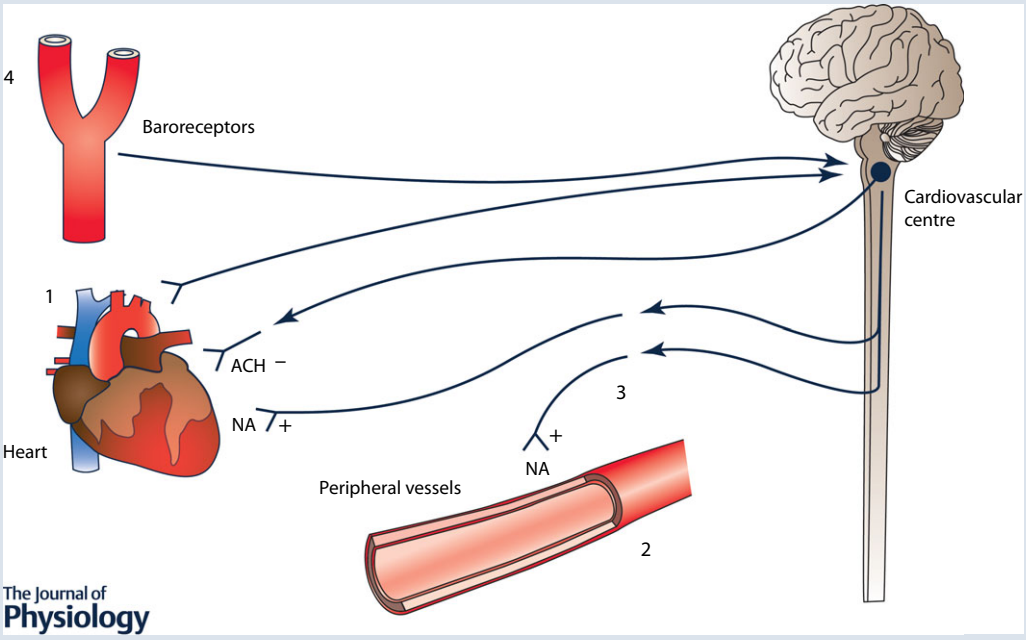

Abstract This review focuses on how to assess autonomic function in humans including various ways to measure heart rate, catecholamines, and sympathetic neural activity. The need to assess autonomic function is paramount in many experimental paradigms because of the following. (1) Autonomic dysfunction is present in common diseases like hypertension, diabetes and heart failure, and the magnitude of this dysfunction is broadly related to morbidity and mortality in these disorders. (2) The relationship between autonomic dysfunction and morbidity and mortality can be causal. (3) Interventions that modulate or reverse autonomic dysfunction can improve outcomes in the affected patients. The techniques discussed are also frequently used to understand

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the autonomic response to sympathoexcitatory manoeuvres like exercise, the cold pressor test or mental stress. Because these manoeuvres can engage a variety of sensory and efferent pathways, under some circumstances the physiological responses measured by many of the techniques are directionally similar, in others they are divergent. Thus any investigator seeking to study the autonomic nervous system or its contribution to either normal physiology or pathophysiological conditions must carefully balance a number of considerations to ensure that the right technique is used to address the question of interest.

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Abstract figure legend Schematic diagram highlighting how autonomic function can be measured in humans. (1) Heart rate can be measured and heart rate variability calculated to assess vagal tone. Blockade of ACH receptors with atropine eliminates heart rate variability. Blockade of noradrenaline (NA) with β-adrenergic antagonists has little effect on heart rate variability; hence it is largely seen as an index of vagal tone. (2) Plasma NA concentrations and spillover can also be measured to assess sympathetic activity. (3) Peripheral sympathetic activity to skin and muscle can be measured directly with microneurography. (4) Manoeuvres that influence baroreceptor discharge can be used to evoke changes in heart rate and peripheral sympathetic activity to understand the dynamic components of key autonomic responses. Depending on issues related to experimental design, these techniques can be used to assess responses to various sympathoexcitatory stressors, and gain insight into physiological regulation.

This brief review on how to assess autonomic function in humans is based on my presentation at the second 'UCLA Autonomic Nervous System Control of the Heart in Health and Disease Symposium' held in 2015. The fundamental ideas underpinning the talk are threefold. First, autonomic dysfunction is present in many common diseases like hypertension, diabetes and heart failure, and the magnitude of this dysfunction is broadly related to morbidity and mortality in these disorders. Second, the relationship between autonomic dysfunction and morbidity and mortality can be causal. Third, efforts via lifestyle (especially exercise training), pharmacological, or device interventions to modulate or reverse autonomic dysfunction should then generally improve outcomes in the affected patients. With this perspective as a background I will start with simple measures of heart rate and move to more complex and invasive techniques like microneurography to measure sympathetic neural discharge in humans, and noradrenaline spillover to provide an index of whole body or regional sympathetic activity. In general the more invasive techniques beyond heart rate are only available in specialized research labs and are not used clinically. An important caveat to this effort is that the literature cited will be selective to either reinforce a point or direct the interested reader to more comprehensive review articles.

Heart rate

Heart rate is perhaps the simplest measure of autonomic function, and interest in its measurement and 'pulsology' predates technological medicine in a number of traditions (Chamsi-Pasha & Chamsi-Pasha, 2014). It is also the one index of autonomic function that can be measured easily, accurately and unambiguously and is thus suitable for large population-based or epidemiological trials.

On a population basis high resting heart rate is generally associated with higher morbidity and mortality in both unselected populations and in patients with underlying diseases (Cook *et al.* 2006; Zhang *et al.* 2015). Additionally, in subjects followed over time a marked increase in resting heart rate is linked to an increased risk of death from ischaemic heart disease and all-cause mortality (Nauman *et al.* 2011). Finally, a slow heart rate recovery after exercise is also associated with increased mortality (Cole *et al.* 1999).

From a mechanistic perspective administration of high doses of β-adrenergic blocking drugs causes only a small (5–10 beat) reduction in resting heart rate while blockade of muscarinic receptors with atropine causes a much larger ($\sim$40 beat) increase in resting hear rate (Martin *et al.* 1974). Such observations have led to the concept that resting heart rate is typically dominated by vagal tone and that loss of this tone has pathophysiological implications perhaps because it normally can suppress life threatening arrhythmias (Smith *et al.* 2005).

Heart rate variability

Like heart rate, heart rate variability can have a strong relationship with morbidity and mortality (Ponikowski *et al.* 1997; La Rovere *et al.* 2003; Hemingway *et al.* 2005). Heart rate variability is assessed by measuring beat to beat changes in heart rate (Agelink *et al.* 2001). There are a number of statistical indices that can be derived from these measurements but because atropine attenuates

or abolishes most of them they are generally thought to reflect vagal tone (Toska & Eriksen, 1993; Yamamoto & Hughson, 1994). This interpretation is based on the differential effects of muscarinic *vs.* β-adrenergic blockade on heart rate variability (HRV) at this frequency; the effects of muscarinic blockade are much more dramatic than β-adrenergic blockade.

Either lower baseline levels or loss of variability at this frequency is associated with poor outcomes for both cardiovascular and all-cause mortality. Exercise training can prevent the age-related decline in heart rate variability and restore it in those with low values (Sandercock *et al.* 2005). Of note, low dose administration of muscarinic antagonists can have paradoxical vagotonic effects that also restore vagal tone in diseases like heart failure, but large-scale definitive randomized clinical trials of such therapy have been limited and the small-scale trials have not been judged to be promising (La Rovere *et al.* 1994). Finally, there is controversy in the literature about the extent to which heart rate variability is driven by subtle respiration-associated changes in blood pressure, pulmonary stretch reflexes or via central mechanisms that link vagal tone and respiratory motor output in the brainstem (Eckberg, 2000). However, whatever the driving mechanisms, my perspective based on the autonomic blocking studies noted above is that HRV largely reflects changes in vagal tone. That HRV is linked to outcomes in a number of conditions (irrespective of mechanism) is an essential point when considering its utility as a test of autonomic function. Because the mechanisms behind HRV and the role of vagal tone as a major driver of it are contentious issues, additional ideas and historical context can be found in a number of outstanding papers (Pomeranz *et al.* 1985; Kollai & Mizsei, 1990; Jokkel *et al.* 1995; Schlafke, 1995).

Plasma catecholamines

Plasma catecholamines are relatively easy to measure, and can also be linked to outcomes. They also rise during sympathoexcitatory physiological stressors like exercise and track other indices of sympathoexcitation (Seals *et al.* 1988). A key caveat is that the term sympathoexcitatory is an oversimplification and commonly used manoeuvres like whole body exercise, handgripping, the cold pressor test, mental stress, hypoxia and/or hypercapnia differ in the sensory and efferent systems they engage. Extensive discussion of the physiological nuances of each class of manoeuvres is beyond the scope of this review, but care must be used in the design and interpretation of experiments depending on the stressor used and outcomes measured.

Perhaps the most notable example of this linkage is the relationship between plasma catecholamines (especially noradrenaline) and outcomes in congestive heart failure (Cohn, 1995). Observations that higher levels of noradrenaline were associated with worsening outcomes in heart failure also underpin the highly successful (and initially controversial) use of β-blocking drugs in heart failure (Bristow, 2000). Of note, exercise training interventions appear useful in blunting the so-called neurohumoral activation in congestive heart failure (Gademan *et al.* 2007).

The main limitation of plasma noradrenaline (and also adrenaline) in the evaluation of autonomic function is that it represents a snap shot of activity (unless serial measurements are made). Additionally, values measured in the plasma reflect the net balance of neural release, neural reuptake and other forms of clearance. For example, under some circumstances like acute hypoxia, there can be clear increases in sympathetic neural activation, but limited increases in plasma noradrenaline (Rowell *et al.* 1989).

Noradrenaline spillover

Using radioactive tracer technology it is possible to overcome many of the limitations associated with the simple measures of plasma catecholamines including those related to reuptake and clearance (Meredith *et al.* 1993). It is also possible to make both whole body measurements and measurements across vascular beds that perfuse a given organ, for example the kidney. The main limitation to the widespread application of these measurements is that they are invasive and require multiple catheters in various locations in the vasculature, and the use of radioactive material can pose logistical and regulatory challenges at some institutions. However, these techniques essentially have no rival or alternative for the measurement of regional sympathetic activity to areas not accessible to direct neural recording. With the exception of skeletal muscle and skin, such direct recording techniques are limited by technical and ethical considerations in humans.

Microneurography

In humans, as noted above, it is possible to use microelectrodes to measure sympathetic neural activity to muscle and skin peripheral nerves (Charkoudian & Wallin, 2014). The nerves used for measurement innervate limbs and thus contain a mixture of motor, sensory and sympathetic nerves and are typically close to the surface of the body. The most frequently used site being the common peroneal nerve on the lateral aspect of the leg near the knee. The history of how this technique was developed and how it emerged in part by accident from efforts to study motor function in humans is also an object lesson in scientific serendipity (Vallbo *et al.* 2004).

As is the case with heart rate, heart rate variability, plasma catecholamines and spillover measurements,

muscle sympathetic nerve activity (MSNA) is also deranged in conditions like congestive heart failure (Leimbach *et al.* 1986). However, the technical difficulty of the technique means that it has not been used in large outcome studies. Other limitations of microneurography include its snap-shot measurement window, need for a still subject, and concerns about how measurements made in skin or muscle reflect other organs. However, for a given subject the MSNA is highly reproducible, but there is wide (5- to 10-fold) subject to subject variability and the relationship between MSNA and blood pressure is dependent on the subject's age and sex (Joyner *et al.* 2015). In middle aged and older subjects there is a direct relationship between baseline MSNA and blood pressure with the relationship being stronger in women than men. Additionally, while it has been difficult to directly link higher levels of MSNA to blood pressure, the fall in blood pressure during ganglionic blockade is directly related to baseline levels of MSNA and also plasma noradrenaline (Jones *et al.* 2001; Barnes *et al.* 2014). Unfortunately, the drugs needed to routinely perform ganglionic blockade in humans are no longer available.

In contrast to MSNA, skin sympathetic activity is highly variable because it is temperature dependent. Additionally, the sympathetic nerves to the skin control both sweating and skin blood flow and the skin contains sympathetic dilator nerves. Thus both vasoconstriction and vasodilatation in most areas of human skin can be neurally mediated (Johnson *et al.* 2014). Thus, in addition to its technically demanding nature, there are many caveats that need to be appreciated to successfully use microneurography to study the sympathetic nervous system in humans.

Summary and perspective

In this brief summary, I have emphasized that alterations in autonomic function are broadly associated with both increased cardiovascular and in many cases all-cause mortality in humans. Each technique to assess autonomic function outlined above has strengths and weaknesses. They also vary by several orders of magnitude in complexity, invasiveness and the degree of logistical support required for their successful application and interpretation. These techniques are also frequently applied during manoeuvres like tilting, baroreflex testing, exercise and various other tests of sympathoexcitation (e.g. mental stress). Under some circumstances the 'answers' generated by any of the techniques are generally or directionally similar; in others they are divergent. This means that any investigator seeking to study the autonomic nervous system or its contribution to either normal physiology or pathophysiological conditions must carefully balance a number of considerations. Foremost are the questions being asked and then the experimental design and interventions needed to address them. For large outcome studies and clinical trials, measurements of MSNA or noradrenaline spillover might be intellectually ideal but impossible in a practical sense. This general problem was recently highlighted in the 'failed' phase III clinical trial of catheter-based renal denervation for resistant hypertension (Bakris *et al.* 2014). Part of this failure might have been because there was no easy way to select patients who clearly had resistant hypertension driven by high sympathetic activity and because there was no easy way to assess the efficacy of the ablation (Joyner, 2014). This experience stresses the ongoing need to assess autonomic function in humans especially in so-called translational research. It also highlights the many challenges associated with making these measurements and translating laboratory-based insights to human clinical trials.

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Additional information

Competing interests

None declared.