The measurement of autonomic function in clinical practice

M. J. BROWN, MSc, MD, FRCP
Professor of Clinical Pharmacology, Addenbrooke's Hospital, Cambridge

The importance of the sympathetic nervous system in cardiovascular regulation has led clinical researchers to invest much effort into the development of methods for measuring its activity in healthy subjects in response to a variety of stimuli, and in a variety of disease states. By contrast, there has to date been only limited applications for the sophisticated biochemical and other techniques that are now everyday tools in clinical research. This article reviews the main clinical applications of autonomic functions tests, together with the techniques that are commonly employed.

The measurement of sympathetic activity

The sympathetic nerves are the one part of the nervous system whose activity can be assessed (within certain limitations) by the biochemical estimation in plasma of their neurotransmitter, noradrenaline. Unlike acetylcholine, which is the neurotransmitter of both the voluntary nervous system and the parasympathetic nerves, noradrenaline has a relatively long duration of action in the synaptic clefts, where it is released, and subsequently in the bloodstream. Putative purinergic transmitters, such as ATP, are also rapidly metabolised; whereas several of the putative peptide neurotransmitters may be too large to diffuse from nerve endings in the adventitia of blood vessels to the vessel lumen.

However, it is important to realise that the noradrenaline circulating in the bloodstream is almost entirely a spillover from nerve endings and does not itself, under normal circumstances, contribute to the effects of sympatho-adrenal activation. Moreover, it has become clear over the past few years that the proportion of circulating noradrenaline arising from different tissues varies not with the density of sympathetic innervation in a tissue but with the width of the synaptic gap [1–3]. This is because noradrenaline released into a narrow synaptic gap is recaptured by the nerve ending, whereas in wider clefts the noradrenaline is more likely to spill into the circulation. In particular, the heart contributes a surprisingly small fraction of a measured plasma noradrenaline concentration—probably less than 5 per cent. At the same time, it has become clear that sympathetic activity to individual tissues can vary independently, so that, for instance, increased renal sympathetic nerve activity may contribute to some cases of renovascular hypertension where plasma renin is not elevated—but plasma noradrenaline also lies within the normal range.

Noradrenaline is often measured together with its *N*-methylated metabolite, adrenaline, and it is useful to consider briefly the origins of the latter before describing the measurement techniques themselves. Adrenaline is the major catecholamine secreted by the adrenal medulla. This is because the enzyme which catalyses its synthesis from noradrenaline is absolutely dependent on induction by high concentrations of glucocorticoids, which are present within the adrenal medulla as a result of the portocapillary circulation from the cortex. Unlike noradrenaline, adrenaline is a true circulating hormone, but its functions in man have been largely subsumed by the sympathetic nervous system, and under most physiological conditions the concentration of adrenaline in the bloodstream is too low to contribute to the effects of sympathoadrenal activation. The exceptions relate to the relative $beta_2$: $beta_1$ receptor selectivity of adrenaline (compared to noradrenaline). The major effect of $beta_2$ stimulation appears to be a reduction in plasma potassium concentration secondary to potassium influx into cells [4–6]. This may play a protective role during severe exercise, but may contribute to arrhythmias in patients suffering a myocardial infarct. The other characteristic of adrenaline, which differentiates it from other physiological hormones, is that its release is not subject to any negative feedback control but can to the contrary be enhanced by a number of amplification loops (eg via ACTH and angiotensin-II release); this may explain why several hundred-fold increases in the plasma concentration of adrenaline can occur rapidly *in extremis* (eg severe shock), and suggests that adrenaline is the final emergency limb of the 'fight and flight' reaction [7].

Measurement techniques

Two main types of technique exist for the measurement of noradrenaline and adrenaline: in addition, several of their metabolites can be measured by simpler methods in most routine biochemistry laboratories.

The radioenzymatic assay of catecholamines is based on their specific conversion to a radioactively labelled methylated product in the presence of the enzyme, catechol-*O*-methyltransferase (COMT), and a radioactively labelled methyl donor, *S*-adenosyl-L-methionine. This technique is the most sensitive available and can be made very precise by use of a double-isotope technique to

Address for correspondence: Professor M. J. Brown, Clinical Pharmacology Unit, Level 2, F&G Block, Addenbrookes Hospital, Hills Road, Cambridge CB2 2QQ.

correct for the variable recovery of label in different samples. The main problem is one of cost since saturating amounts of label are used to ensure that the rate of enzymatic conversion of the catecholamine is not limited by the concentration of the methyl donor [8,9].

The alternative technique is high performance liquid chromatography (HPLC) using an electrochemical detector. The principle of this is that catecholamines are readily oxidised by applying a voltage across the effluent from the HPLC column, and the size of the resulting oxidation current is proportional to the concentration of catecholamine injected on to the column. This technique can be semi-automated, and the recently introduced Waters detector ensures detection of noradrenaline in most plasma samples. However, the identification of adrenaline continues to be a problem, with 'spurious adrenaline peaks' leading some patients with suspected phaeochromocytoma to unnecessary investigations and even surgery. I would not currently diagnose a phaeochromocytoma on the basis of an isolated HPLC measurement showing an elevated adrenaline but normal noradrenaline concentration.

It is not reasonable to expect either of the above techniques to be available in most routine laboratories, and these rightly use a simpler method for the major clinical application described below—screening for phaeochromocytoma. Vanillylmandelic acid (VMA) is the product of noradrenaline and adrenaline when they have been metabolised by both COMT (the same enzyme used in the radioenzymatic assay) and monoamine oxidase (MAO)—the enzyme whose inhibition is employed in the therapy of depression. VMA is formed both within nerve endings that capture the circulating catecholamines and within the liver. There is a long time delay between its formation and its appearance in the bloodstream, so that there is no point in trying to measure short-term changes in the circulating concentration and it is in practice measured in a 24-hour urine sample. The most commonly used technique is based on spectrophotometric estimation of the product of VMA oxidation. Providing that the laboratory concerned is given enough samples to be running the method regularly, the technique is a reliable one, and the only known drug interference is by L-Dopa, whose deaminated and methylated metabolite, homovanillic acid, is structurally similar to VMA.

Clinical applications

As already intimated, chemical applications are limited—in fact to two principal conditions: one with elevated catecholamine secretion, phaeochromocytoma and the other with reduced secretion, autonomic neuropathy.

The diagnosis of phaeochromocytoma

This has been extensively reviewed [10,11]. It is undoubtedly a very rare condition, accounting for less than 1 per cent of all patients with hypertension. On the other hand, it is one of the few truly curable causes of hypertension, and is also potentially a lethal cause—either because of the impossibility of detecting malignancy in advance, or because of spontaneous and catastrophic infarction of the tumour. These reasons justify the routine screening of all but the most elderly of hypertensive patients, since the other clinical features associated with the diagnosis (paroxysmal hypertension, sweating, pallor etc) are frequently absent.

The role of catecholamine and metabolite measurements is much greater, therefore, than the incidence of the tumour itself might suggest, and this role is magnified by the anxiety which is correctly engendered in physicians about missing the diagnosis when the first measurements are borderline. However, a few simple rules of thumb help to reduce this anxiety and hence the need for further tests in the majority of patients with borderline VMA estimations.

1. It is useful to divide the diagnostic process into two separate and sequential parts. The first, resting entirely on biochemical tests, answers the question, does the patient have a phaeochromocytoma? The second, mainly radiological, answers the question, where is the tumour? False positive and negative diagnoses arise when the localising tests are used to try and answer the first question.

2. Patients with a phaeochromocytoma are relatively insensitive to their elevated catecholamine levels, this being a good example of receptor down-regulation. Thus, even mild degrees of hypertension require a several-fold elevation of plasma catecholamine values, if the latter are the cause of the hypertension. Therefore a trivial (less than two-fold) elevation of VMA excretion in a patient with severe or malignant hypertension is evidence against the diagnosis of phaeochromocytoma—especially if (as is often the case) the patient is being treated with a vasodilator drug that itself causes baroreflex activation of noradrenaline release.

3. Only a minority of phaeochromocytoma patients have plasma catecholamine values that are less than two-fold elevated, and above this range only cardiac failure of a clinically apparent degree can commonly cause a similar elevation of catecholamine values [12]. Even in patients who are intermittently normotensive, it is exceptionally rare for both plasma catecholamines to be within the normal range; I have not found this in over 50 patients.

4. Large tumours secrete mainly noradrenaline because most of the cells (even in the adrenal) are no longer exposed to a high cortisol concentration that induces adrenaline synthesis. A significant degree of hypertension requires the elevation of noradrenaline.

As well as confirming the diagnosis, the measurement of plasma catecholamines is useful in indicating the likelihood of an adrenal site of the tumour, in which case (comprising 90 per cent of the tumours) plasma adrenaline levels are usually raised. If only the adrenaline concentration is found to be high, and this is confirmed by radioenzymatic assay, or if the plasma noradrenaline concentration is less than twice normal, the blood sampling should be repeated before and after pentolinium [13]. This is a ganglion-blocking drug which suppresses physiological but not tumour-derived secretion of catecholamines, although suppression is not always seen if the basal values are not themselves elevated. We use a 2.5 mg

intravenous bolus of pentolinium, with blood samples being drawn immediately before and at 10 and 20 minutes post-pentolinium. The drug should not be given to patients with significant renal impairment, and patients should remain supine for 90 minutes after injection of pentolinium.

Localisation of the tumour. This aspect is not strictly relevant to this article, except that in approximately 15 per cent of patients with extra-adrenal phaeochromocytomas, the radioisotope (mIBG) scan fails to locate the tumour and it is necessary to perform selective venous sampling [14,15]. This technique was once used by us for all patients prior to the advent of CT scanning for adrenal tumours [16] and then radioisotope scanning for the extra-adrenal tumours [17]. Probably, experience of which samples are necessary at the time of the sampling procedure, together with the laboratory backup, are necessary for the technique to remain the ultimate localising method, and it would seem unadvisable to undertake this outside a specialised centre [18]. It is not yet apparent which tumours fail to accumulate the mIBG isotope, although one sub-group is the chemodectomas (carotid body and glomus jugulare tumours) which arise from chemoreceptor tissue that may lack the noradrenaline uptake pump by which mIBG is accumulated in chromaffin tissue [19].

Autonomic neuropathy

Unlike phaeochromocytoma, this is a clinical syndrome with several causes, and the precise clinical features in an individual patient depend on which parts of the autonomic nervous system—parasympathetic and sympathetic, afferent and efferent nerves—are involved. Again, there have been numerous reviews [20,21] so what follows will be a slightly personal view of how these patients can be diagnosed most efficiently using modern techniques for assessment of sympathetic and parasympathetic activity.

Whereas, in phaeochromocytoma the excess circulating levels of catecholamines arise from 'endocrine' secretion from the adrenal gland (or an ectopic tumour), in autonomic neuropathy it is usually degeneration of the sympathetic nerves that leads to clinical problems. Given the variable relation between circulating and synaptic cleft noradrenaline concentrations, it is not surprising that single plasma noradrenaline estimations are not useful in making the diagnosis except in very severe cases. Even when the adrenal medulla is also degenerate, the wide range of 'normal' plasma adrenaline levels can make it difficult to diagnose adrenomedullary failure from a single resting sample. So in the case of both catecholamines the diagnosis of autonomic neuropathy requires the measurement of their response to stimulation.

Reviews of autonomic neuropathy usually list a formidable battery of tests used in the assessment of patients. Even the apparently simple ones, such as the Valsalva manoeuvre or measurement of respiratory variation in heart rate, are not easy to perform with sufficient accuracy to be helpful unless performed with the aid of equipment that can provide a continuous record of blood pressure or heart rate. The loss of respiratory variation in heart rate is often the earliest, and therefore the most sensitive indicator of autonomic neuropathy, and is due to vagal nerve degeneration[22]. However at this stage patients are likely to be asymptomatic so that the test is helpful clinically only in differentiating autonomic neuropathy from other causes (such as salt depletion). For researchers, clearly, it has greater use in drawing their attention to patients with autonomic neuropathy before they present with the clinical problem of orthostatic hypotension.

Since orthostatic hypotension is the main clinical problem, and is due to a failure of the normal sympathetic activation in response to standing, the most direct and simplest method of evaluating such patients is to measure the plasma noradrenaline concentration in the supine position and after standing for two and five minutes. In patients who cannot stand at all, the test can be performed using a tilt table to raise the patient to only 45°. As always with samples for catecholamine analysis, the blood should be taken into chilled lithium heparin tubes and separated as soon as possible. Thereafter the plasma must be kept frozen and transported on dry ice (or in liquid nitrogen) to the catecholamine laboratory. Providing that the samples can be stored at $-70°C$, the catecholamines are very stable. The normal erect plasma noradrenaline concentration varies between 30 and 100 per cent above the supine level.

The other form of stimulation test which is occasionally useful in diabetics with hypoglycaemic episodes, is to induce hypoglycaemia and measure the adrenaline response. This is one circumstance where adrenaline is more important than noradrenaline (which may not be released at all in response to hypoglycaemia). However, patients can still mount a counterregulatory response to hypoglycaemia in the face of adrenomedullary failure if their glucagon secretion is intact, and the evaluation of counterregulation requires at a minimum the measurement of adrenaline and glucagon. It may still be felt to be useful to determine whether the lack of appropriate warning symptoms in response to hypoglycaemia is due to adrenomedullary failure, but the management of such patients is generally guided more by their clinical than their biochemical features.

Assessment of treatment. The measurement of catecholamines plays a surprisingly small part in the assessment of the treatment of autonomic neuropathy. This is partly because the indirect sympathomimetics that have been used in an attempt to increase noradrenaline release have not proven useful; and partly because of increasing realisation that the hypotension is often compounded by fluid depletion, and treatment can more usefully be focussed on this area than directly on the sympathetic nervous system[21,23]. Patients with autonomic neuropathy have reversed day/night patterns of urinary flow, possibly because renal perfusion is improved when they are supine at night. Symptomatic improvement can

sometimes be obtained if patients sleep in a semi-recumbent position. Drug therapy now concentrates on the use of fludrocortisone to enhance sodium retention; and recently the use of a nocturnal dose of DDAVP has been advocated to stimulate nocturnal urinary concentration [24]. However, the latter must be used cautiously, and only after a first test dose, if water intoxication is to be prevented.

Denervation hypersensitivty. Just as patients with phaeochromocytoma have down-regulated receptors, patients with autonomic neuropathy are hypersensitive to catecholamines. This is usually a pharmacological curiosity, although from a research point of view these patients may be very valuable in helping to establish whether some of the putative co-transmitters of noradrenaline (ATP and neuropeptide Y) play a physiological role. Occasionally, we have seen patients in whom the denervation hypersensitivity itself leads to clinical problems. The most striking example was a patient with the Holmes-Adie syndrome who presented with paroxysmal headaches and hypertension, and on the basis of a spurious HPLC catecholamine estimation was extensively investigated for phaeochromocytoma. This patient had normal adrenaline release but reduced noradrenaline release, and the hypertension was due to stimulation by adrenaline of an increased number of alpha-adrenoceptors. Normally, adrenaline cannot raise mean arterial pressure since the rise in systolic is offset by the $beta_2$-receptor mediated fall in diastolic pressure. The $beta_2$-receptor, however, is not innervated and therefore not subject to denervation hypersensitivity. Pharmacologically speaking, the selective hypersensitivity of the alpha-receptor converts adrenaline to noradrenaline.

Conclusion

Clinical problems associated with either excessive or reduced catecholamine secretion are uncommon, but are usually treatable and therefore worth considerable diagnostic effort. Using available drugs and analytical techniques, the clinical pharmacologist is able to learn in most patients the site of the lesion, while both phaeochromocytoma and autonomic neuropathy patients will continue to teach us about further aspects of the autonomic system.

References

1. Brown, M. J., Jenner, D. A., Allison, D. J. and Dollery, C. T. (1981) *Clinical Science,* **61,** 585.
2. Esler, M., Blombery, P., Leonard, P. *et al.* (1982) *Clinical Science,* **63,** 285.
3. Bevan, J. A. (1978) *Federation Proceedings,* **36(10),** 2439.
4. Brown, M. J., Brown, D. C. and Murphy, M. B. (1983) *New England Journal of Medicine,* **309,** 1414.
5. Struthers, A. D., Reid, J. L., Whitesmith, R. and Rodgers, J. C. (1983) *British Heart Journal,* **49,** 90.
6. Clausen, T. and Flatman, J. A. (1980) *British Journal of Pharmacology,* **68,** 749.
7. Editorial (1985). *Lancet,* **i,** 561.
8. Da Prada, M. and Zurcher, G. (1976) *Life Science,* **19,** 1116.
9. Brown, M. J. and Jenner, D. A. (1981) *Clinical Science,* **61,** 591.
10. Manger, W. M. and Gifford, R. W. (1977) *Pheochromocytoma.* New York: Springer-Verlag.
11. Brown, M. J. and Dollery, C. T. (1981) *Update,* **23,** 1237.
12. Cohn, J. N., Levine, T. B., Olivari, M. T. *et al.* (1984) *New England Journal of Medicine,* **311,** 819.
13. Brown, M. J., Allison, D. J., Jenner, D. A. *et al.* (1981). *Lancet,* **i,** 174.
14. Editorial (1984) *Lancet,* **ii,** 905.
15. Brown, M. J. and Allison, D. J. (1984) *Lancet,* **ii,** 1274.
16. Stewart, B. H., Bravo, E. L., Haaga, J. *et al.* (1978) *New England Journal of Medicine,* **299,** 460.
17. Sisson, J. C., Frager, M. S., Valk T. W. *et al.* (1981) *New England Journal of Medicine,* **305,** 12.
18. Allison, D. J., Brown, M. J., Jones, D. H. and Timmis, J. B. (1983). *British Medical Journal,* **286,** 1122.
19. Dunn, G. D., Brown, M. J., Sapsford, R. N. *et al.* (1986). *Lancet,* **i,** 1061.
20. Bannister, R. (1983), *Autonomic failure.* Oxford: Oxford University Press.
21. Sever, P. S. (1986) In *Advanced Medicine,* Vol. 21 (ed M. J. Brown). Edinburgh: Churchill Livingstone.
22. Bannister, R., Davies, B., Holly, E. *et al.* (1979) *Brain,* **102;** 163.
23. Davidson, C., Smith, D, and Morgan, D. B. (1976) *American Journal of Medicine,* **61,** 709.
24. Mathias, C. J., Fosbraey, P., Da Costa, D. F. *et al.* (1986) *British Medical Journal,* **293,** 353.