Br. J. Pharmac. (1976), **56**, 271–277

BLOOD PRESSURE RESPONSES TO ADRENAL FIELD STIMULATION AS A MEASURE OF ADRENAL CATECHOLAMINE RELEASE

D.E. CLARKE & W.M. ROMANYSHYN

Department of Pharmacology, Division of Autonomic Neurochemistry, University of Houston, Houston, Texas 77004, U.S.A.

1 A new method for studying adrenal medullary release is described in pithed rats using field stimulation of an entire adrenal gland.

2 The increases in blood pressure in response to field stimulation of the gland consisted of an initial short, variable component, due to stimulation of adrenergic vasomotor neurones and a secondary, longer lasting component, due to medullary catecholamine release.

3 Removal of the initial component by drugs or cardiac/coeliac ganglionectomy did not affect the magnitude of the secondary pressor component.

4 The secondary pressor component was frequency-dependent, reproducible and stable with time. The extent of medullary catecholamine release could be assessed by comparing the blood pressure rise with those obtained after adrenaline injections.

5 The method appears to provide a reliable means by which adrenal medullary catecholamine release may be assessed without significant interference from vasomotor nerves.

Introduction

The systemic blood pressure rise in response to splanchnic nerve stimulation has been used frequently to assess the activity of pharmacological agents on adrenal catecholamine release. However, splanchnic nerve stimulation also evokes a neuronally mediated vasoconstrictor response in the splanchnic vascular bed which forms a considerable component of the blood pressure response in the rat. In adrenalectomized rats with grafts of adreno-cortical tissue (Carpi & Cartoni, 1968) the blood pressure responses to low frequency splanchnic nerve stimulation remained unaltered, while at higher frequencies, they were still 60 to 65% of that obtained in the intact control rats.

In the present work the blood pressure response to direct electrical stimulation of the whole adrenal gland of pithed rats was analyzed for its usefulness as an indicator of adrenomedullary hormone release without interference by vasoconstrictor neurones. The availability of such a method could be important for the evaluation of adrenomedullary function. Extensive biochemical evidence has accumulated concerning drug-induced and hormonal effects on enzymatic activity in the adrenal medulla (Thoenen, Mueller & Axelrod, 1969; Kirshner, 1969; Mueller, 1971; Stjärne, 1972; Wurtman, Pohorecky & Baliga, 1972; Dairman, 1973; Costa & Guidotti, 1973), but correlations with the ability of the medulla to release catecholamines have generally not been made.

Methods

Male albino Sprague-Dawley rats (200–250 g) were pretreated with atropine (1 mg/kg, i.p.) and anaesthetized with pentobarbitone sodium (60 mg/kg, i.p.). After insertion of a tracheal cannula, the animals were pithed, and maintained on artificial respiration (Phipps & Bird, Model 71216) as described previously (Clarke, 1970). Blood pressure was recorded from a carotid artery, with a Statham (P23Dc) pressure transducer and a Narco Bio-Systems (DMP4a) physiograph and heart rate was measured with a Narco Bio-Systems biotachometer coupler (7302). The right femoral vein was cannulated for the injection of drugs, and both heparin (500 units/kg) and tubocurarine (1 mg/kg) were administered. A transverse incision was made in the ventral abdominal wall, just below the thorax, and the viscera were carefully retracted in order to expose the left adrenal gland. All bleeding was stopped with a cautery and the exposed liver and intestine were covered with saline-soaked gauze pads. The connective tissue and excessive fat surrounding the adrenal gland were cautiously teased away, avoiding damage to the vascular supply. Bipolar platinum electrodes (Palmer) were placed underneath the gland, which was held firmly on the electrodes by means of two ligatures previously passed through the periadrenal fat. The supramaximal voltage was determined for each preparation, and stimulations of 1 ms duration for 20 s were made over a range of

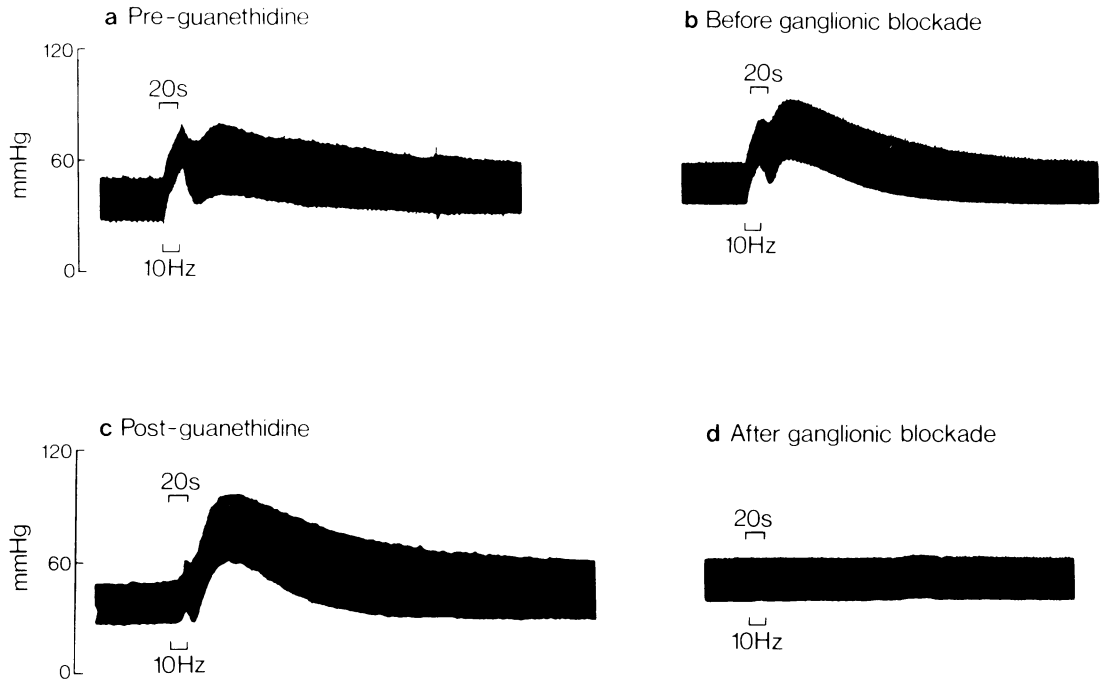

Figure 1 Blood pressure responses to field stimulation (10 Hz, 1 ms, 25 V for 20 s) of the entire left adrenal gland obtained in two pithed, atropine-(1 mg/kg, i.p.) and tubocurarine-(1 mg/kg, i.v.) treated, rats. (a) and (b) Controls; (c) guanethidine (5 mg/kg, i.v.) 2 h previously; (d) chlorisondamine (2 mg/kg, i.v.) 10 min before stimulation.

frequencies (0.5 to 20 Hz), with a Grass Instruments (SD5) stimulator. The peak rise in systolic blood pressure following stimulation, or adrenaline injection was measured.

Adrenal demedullated and sham-operated control rats were obtained from Zivic-Miller Laboratories, Inc. (Allison Park, PA). The animals were kept at 22°C on a diet of pellets (Purina Laboratory Chow) and given 0.9% w/v NaCl solution (saline) as the sole drinking fluid.

Drugs

The following drugs were used: adrenaline hydrochloride (Parke, Davis & Co.); chlorisondamine (Ciba); hexamethonium bromide (Sigma); guanethidine monosulphate (Ciba); reserpine (Ciba); 6-hydroxydopamine hydrobromide as base (Regis); tetracaine hydrochloride (Winthrop); procaine hydrochloride (Mallinckrodt); pentobarbitone sodium (Sigma); atropine sulphate (Mallinckrodt); tubocurarine chloride (Squibb); (±)-propranolol hydrochloride (Ayerst); phentolamine mesylate (Ciba); physostigmine salicylate (Mallinckrodt); sodium heparin (Riker); edrophonium chloride (Roche); bethanidine sulphate (Burroughs Wellcome); neostigmine bromide (Sigma).

Results

Figure 1 illustrates blood pressure responses following stimulation of the entire left adrenal gland of the pithed rat. Panels a and b show that a biphasic response was obtained. The magnitude of the initial pressor response was found to vary from one experiment to another. In fact, Figure 1a and 1b illustrates two of the larger responses obtained. In some experiments the response was very slight (2 to 5 mmHg), but it was always present. The amplitude of the second component was reproducible and longer lasting. Figure 1c shows that the injection of guanethidine (5 mg/kg, i.v.) diminished the initial component thus demonstrating the involvement of adrenergic neurones in this phase of the total response. Ganglionic blockade with chlorisondamine (2 mg/kg, i.v.) abolished both components (Figure 1d). This indicates the pre-synaptic nature of the total response. In order to check that the dose of chlorisondamine used did not

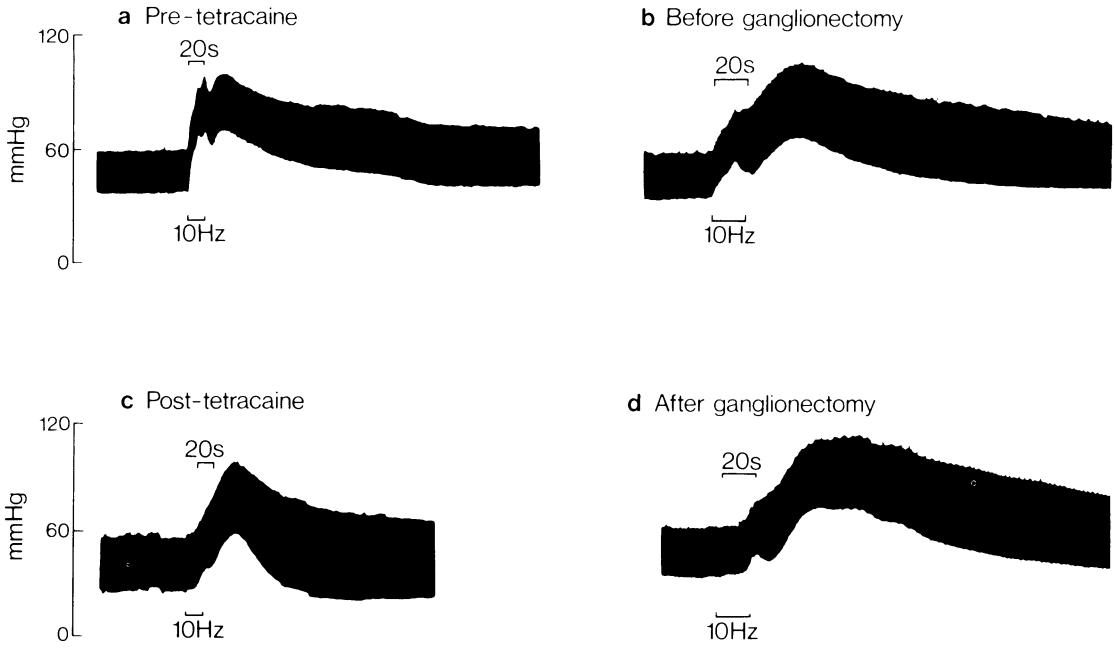

Figure 2 Blood pressure responses to adrenal field stimulation (10 Hz, 1 ms, 20 V for 20 s) of the entire left adrenal gland obtained in two pithed, atropine-(1 mg/kg, i.p.) and tubocurarine-(1 mg/kg, i.v.) treated, rats. (a) and (b) Controls; (c) after local application of tetracaine to the region of the cardiac and coeliac ganglia; (d) after surgical removal of the ganglia.

block postganglionic sympathetic fibres, the adrenergic innervation to the mesenteric vasculature was stimulated distal to the coeliac ganglion. Chlorisondamine failed to attenuate the resulting rise in systemic blood pressure.

Heart rate increases were measured in some experiments. Tachycardia was found to correlate with the onset and progression of the secondary response, suggesting that this component probably represents adrenal catecholamine release.

Selective inhibition of the initial component was obtained following the local application of procaine or tetracaine to the region of the prevertebral ganglia or by surgical removal of the cardiac and coeliac ganglia (Figure 2). A comparison of the frequency-response curves to adrenal stimulation (Figure 3) shows that no

Table 1 The effect of various drugs upon the blood pressure response (second component) to field stimulation of the adrenal gland and to intravenous adrenaline

		Blood pressure response	
Drug	*Dose (mg/kg, i.v.)*	*Adrenal gland stimulation*	*Adrenaline injection (12.5 to 50 ng/300 g body wt.)*
Chlorisondamine	2.0	Blocked	No effect
Hexamethonium	2.0	Blocked	No effect
Phentolamine	2.0	Reversal	Reversal
Propranolol	1.0	Potentiated	Potentiated
Phentolamine + Propranolol	2.0 + 1.0	Blocked	Blocked
Desipramine	0.05	Potentiated	Potentiated
Cholinesterase inhibitors	0.01–2.0	No consistent effect	No effect

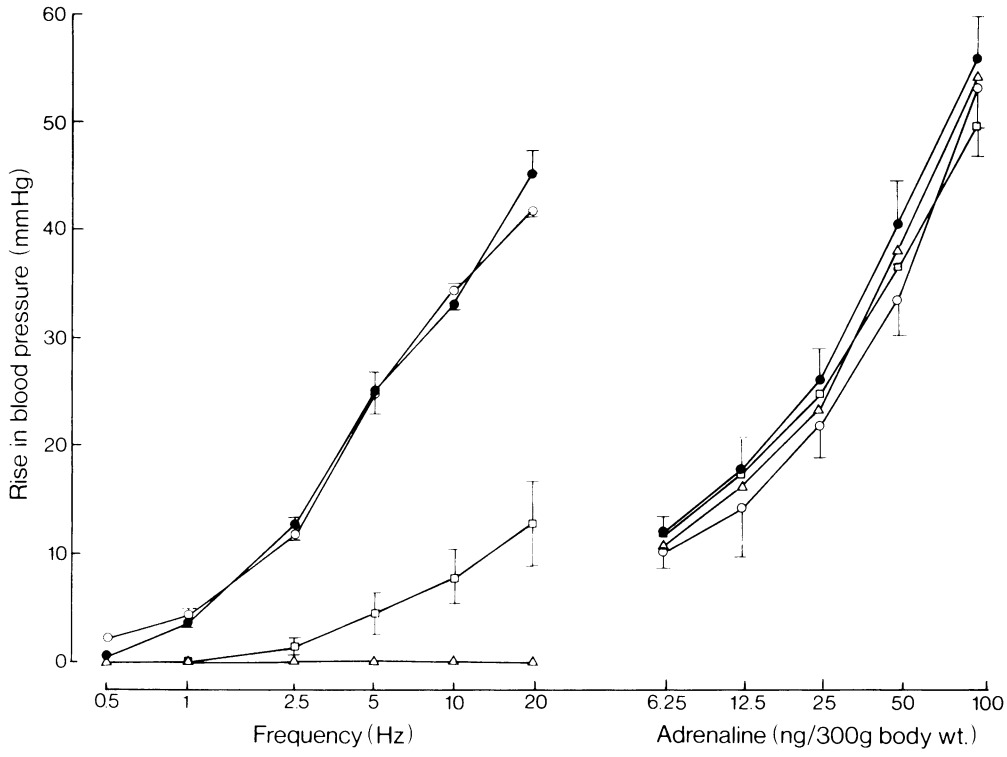

Figure 3 Relation between blood pressure increase (second component) and frequency of adrenal field stimulation (1 ms, 20 V for 20 s) or dose of intravenously injected adrenaline. (○) Control rats; (△) rats treated with chlorisondamine (2 mg/kg, i.v., $n=6$); (□) rats treated with reserpine (10 mg/kg, i.p. 18 h previously, $n=3$); (●) pooled data from rats in which the cardiac or coeliac ganglia were either removed or anaesthetized by local application of tetracaine. All rats were pithed and pretreated with atropine and tubocurarine. Vertical lines show s.e. mean.

significant differences are to be found between conditions where the initial component is present, or inhibited by the use of local anaesthetics or ganglionectomy. Thus the initial component, whether large or small, does not affect the amplitude of the secondary component.

Table 1 summarizes the effect of various drugs on the secondary component of the blood pressure response to adrenal field stimulation and injected adrenaline. Surprisingly, cholinesterase inhibition (with physostigmine, neostigmine or edrophonium) failed to increase the response, despite the indicated pre-synaptic origin. Stimulation of the adjacent body wall or left kidney also failed to evoke any pressor response but stimulation of the peri-adrenal fat after acute unilateral adrenalectomy, gave rise to the initial pressor response only. An identical result was obtained 1 to 2 days after bilateral adrenal demedullation. However, 3 to 10 days later no pressure response could be elicited by adrenal stimulation. The responses to intravenous adrenaline were unimpaired. Stimulation of the right adrenal gland is technically more difficult but in three experiments the blood pressure response obtained under these circumstances did not differ from that described for the contralateral adrenal.

Prolonged stimulation of the gland (10 to 15 min) was conducted. Submaximal frequencies gave rise to a stable hypertensive response with an accompanying tachycardia.

Figure 4 shows the quantitative reliability of the technique over the range of frequencies used. Dose-effect curves to intravenous adrenaline were made to enable a quantitative assay of the response to adrenal stimulation in terms of adrenaline release. Rats showed stable and reproducible responses with time and variation between animals fell within workable limits. This fact is well illustrated in the experiments in which reserpine pretreatment was given (10 mg/kg, i.p. 18 h previously). The frequency-response curve to

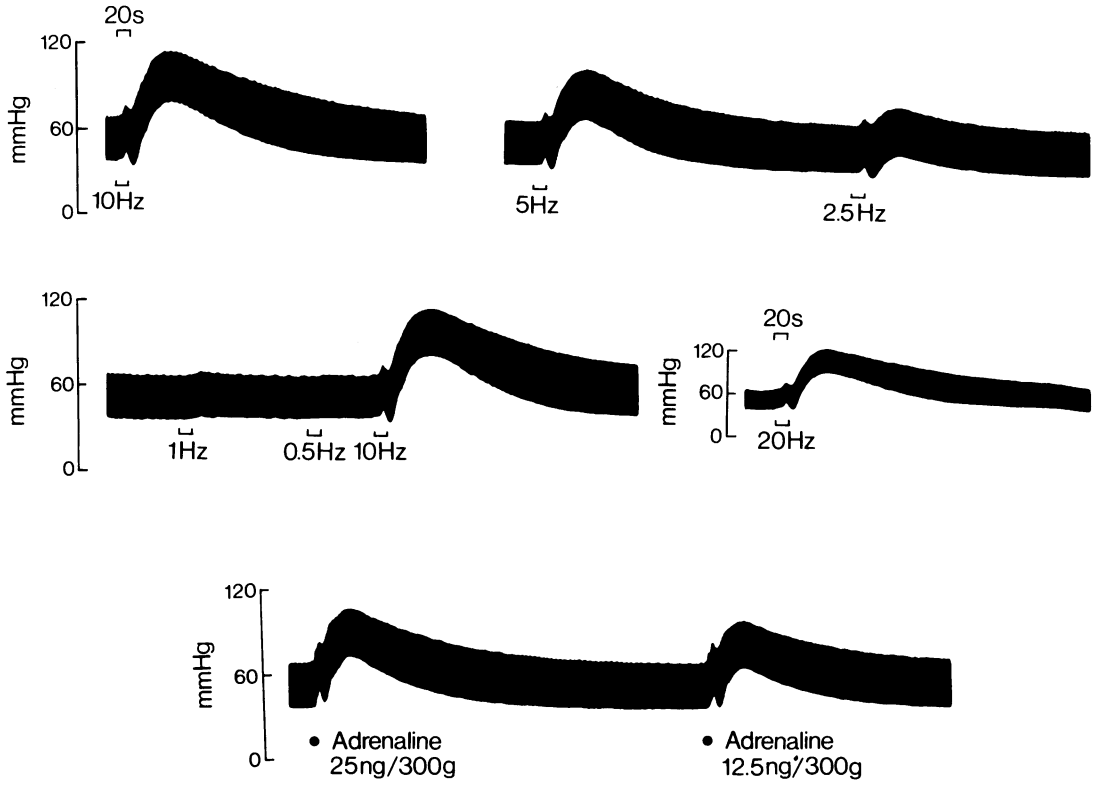

Figure 4 Effect of stimulation frequency on the blood pressure response (second component) to adrenal gland stimulation (1 ms, 25 V for 20 s) in a pithed, atropine-(1 mg/kg, i.p.) and tubocurarine-(1 mg/kg, i.v.) treated, rat given guanethidine (5 mg/kg, i.p., 2 h previously). Note the qualitative similarity to intravenous adrenaline (last two responses).

adrenal stimulation was depressed significantly whereas the dose-effect curve to adrenaline was not altered (Figure 3).

The effects of bethanidine and guanethidine pretreatment (both 5 mg/kg, i.p. 2 h previously), and 6-hydroxydopamine pretreatment (200 mg/kg, i.v. 18 to 22 h previously), were examined. Figure 5 shows that the frequency-response curves were shifted to the left; however, there was a corresponding shift in the dose-effect curves to intravenous adrenaline. These data seem to support the reported inability (Boura & Green, 1965; Thoenen & Oesch, 1973) of these agents to interfere with adrenal catecholamine release and show furthermore, that the extent of the evoked adrenal response can be expressed in quantities of adrenaline injected.

Discussion

Field stimulation of the entire adrenal gland elicited a rise in blood pressure, which consisted of a variable, short, initial component, and a longer lasting secondary component, the amplitude of the latter being dependent on the stimulation frequency. Guanethidine, and other adrenergic neurone blocking agents, abolished selectively the initial component, whereas chlorisondamine and hexamethonium inhibited both components. Presumably, electrical stimulation of the adrenal gland evoked a pre-synaptic excitation of cholinergic neurones leading to a depolarization of adrenergic vasoconstrictor fibres and of adrenal medullary chromaffin cells. These two effects would account for the biphasic blood pressure response. An initial vasoconstrictor component to splanchnic nerve stimulation has been reported in various species (De Vleeschhouwer, 1935; Nickerson & Goodman, 1947; Abercrombie & Davies, 1963), including the rat (Carpi & Cartoni, 1968). These responses would be expected to be fast in onset and short in duration, compared with a response resulting from medullary catecholamine release.

The initial component of the blood pressure response to adrenal field stimulation was shown to be

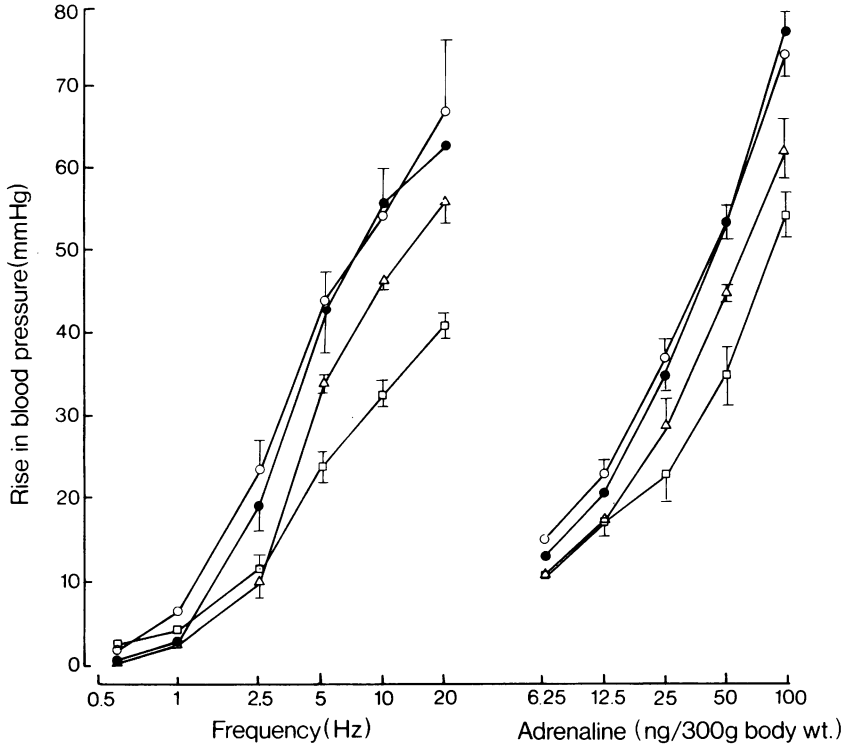

Figure 5 Relation between blood pressure increase (second component) and frequency of adrenal field stimulation (1 ms, 20 V for 20 s) or dose of intravenously injected adrenaline. (□) Control rats; (○) rats pretreated with 6-hydroxydopamine (200 mg/kg, i.v. 18 h previously, $n=3$); (●) rats pretreated with guanethidine (20 mg/kg, i.p., 2 h previously, $n=7$); (△) rats pretreated with bethanidine (20 mg/kg, i.p., 2 h previously, $n=4$). All rats were pithed and pretreated with atropine and tubocurarine. Vertical lines show s.e. mean.

of extra-adrenal origin and entirely dependent upon the functional presence of the cardiac and/or coeliac ganglia. The secondary component closely simulated the blood pressure response to an intravenous injection of adrenaline. It was accompanied by tachycardia and depended upon the presence of the adrenal medulla. Pharmacological characterization of the secondary component also suggests medullary catecholamine release, with adrenaline contributing to a major degree, as shown by the reversal of the response after α-receptor blockade.

The failure of cholinesterase inhibitors to increase the adrenal response to field stimulation cannot be explained at present. The response exhibited either no change or a decrease, irrespective of whether atropine was present or absent. Several possible explanations may be advanced, one possibility is that rat adrenal cholinesterase plays only a minor role in limiting the nicotinic effects of acetylcholine.

The absence of the initial vasoconstrictor component shortly after adrenal demedullation may be a reflection of progressive neuronal degeneration following surgery. This explanation would support the idea that adrenal field stimulation initiated antidromic impulses to the prevertebral ganglia via the splanchnic nerve, rather than inducing pre-ganglionic depolarization through current spread. However, in view of the high voltages used, this latter possibility cannot be ruled out. Some preparations exhibited supra-maximal responses at 4 or 8 V, but 20 to 25 V was always clearly supra-maximal. The encountered differences in the initial component between preparations may be related to variable electrode placement and/or to differing amounts of remaining periadrenal fat. Differing degrees of excitation of extra-adrenal splanchnic fibres is also likely to be involved.

The present experiments have shown that the initial vasoconstrictor response does not quantitatively influence the second component caused by the release of medullary amines. This represents a definite improvement over direct splanchnic nerve stimulation which, in the rat, has been shown to cause wide-spread

neurogenic vasoconstriction (Carpi & Cartoni, 1968). Indeed, guanethidine reduced the response to splanchnic nerve stimulation indicating that the neurogenic vasoconstrictor component was so extensive as to preclude any precise quantitation of the catecholamines released from the adrenal medulla. The present findings show that the technique of adrenal field stimulation allows a more exact evaluation of adrenal catecholamine release. 6-Hydroxydopamine, guanethidine and bethanidine were found not to affect adrenal release as shown previously (Boura & Green, 1965; Thoenen & Oesch, 1973). The enhanced responses to adrenal stimulation found after these drugs were adequately accounted for by the increased responsiveness to injected adrenaline. In addition, responses to adrenaline were unchanged after reserpine but the impairment of adrenal medullary function was readily detectable.

The present paper describes an *in vivo* method for the quantitative assessment of the release of catecholamines from the adrenal medulla without interference from a neurogenic vasoconstrictor component. The method should be of use in studying the acute and chronic effects of drugs, hormones and induced pathological states (e.g. hypertension, hypophysectomy) upon the release of adrenal medullary amines.

This work was undertaken in partial fulfillment of the requirements for the degree of Ph.D. at the University of Houston by W.M.R. Partial support for the work was obtained from Eli Lilly & Company, Indianapolis, Indiana 46206.

References

ABERCROMBIE, G.G. & DAVIES, B.N. (1963). The action of guanethidine with particular reference to the sympathetic nervous system. *Br. J. Pharmac. Chemother.*, **20**, 171–177.

BOURA, A.L.A. & GREEN, A.F. (1965). Adrenergic neurone blocking agents. *Ann. Rev. Pharmac.*, **5**, 183–212.

CARPI, A. & CARTONI, C. (1968). Effects of guanethidine on the blood pressure response to splanchnic nerve stimulation in the rat: role of the adrenal medulla. *Br. J. Pharmac.*, **34**, 259–266.

CLARKE, D.E. (1970). Restoration of tyramine responses by bretylium BW392C60, bethanidine and monoamine oxidase inhibitors in reserpine-treated rats. *Br. J. Pharmac.*, **38**, 1–11.

COSTA, E. & GUIDOTTI, A. (1973). The role of 3′,5′-cyclic adenosine monophosphate in the regulation of adrenal medullary function. In *New Concepts in Neurotransmitter Regulation*, ed. Mandell, A.J. pp. 135–152. New York: Plenum Press.

DAIRMAN, W. (1973). Multiple measures of compensatory adaptation in catecholamine biosynthesis. In *New Concepts in Neurotransmitter Regulation*, ed. Mandell, A.J. pp 1–20. New York: Plenum Press.

DE VLEESCHHOUWER, G. (1935). Au sujet de l'action du diethylaminomethyl-3-benzodioxane (F.883) et du piperidomethyl-3-benzodioxane (F.933) sur le système circulatoire. *Arch. Int. pharmacodyn. Thér.*, **50**, 251–295.

KIRSHNER, N. (1969). Storage and secretion of adrenal catecholamines. In *Advances in Biochemical Psychopharmacology*, ed., Costa, E. & Greengard, P., Vol. 1. pp. 71–89. New York: Raven Press.

MUELLER, R.A. (1971). Effect of 6-hydroxydopamine on the synthesis and turnover of catecholamines and protein in the adrenal. In *6-Hydroxydopamine and Catecholamine Neurons*, ed. Malmfors, T. & Thoenen, H. pp. 291–302. London: North-Holland.

NICKERSON, M. & GOODMAN, L.S. (1947). Pharmacological properties of a new adrenergic blocking agent: N,N-dibensyl-chloroethylamine (Dibenamine). *J. Phamac. exp. Ther.*, **89**, 167–185.

STJÄRNE, L. (1972). The synthesis, uptake and storage of catecholamines in the adrenal medulla. Effects of drugs. In *Catecholamines*, ed. Blaschko, H. & Muscholl, E. pp. 231–269. Berlin: Springer-Verlag.

THOENEN, H., MUELLER, R.A. & AXELROD, J. (1969). Increased tyrosine hydroxylase activity after drug-induced alteration of sympathetic transmission. *Nature, Lond.*, **221**, 1264.

THOENEN, H & OESCH, F. (1973). New enzyme synthesis as a long-term adaptation to increased transmitter utilization. In *New Concepts in Neurotransmitter Regulation*, ed. Mandell, A.J. pp. 33–52. New York: Plenum Press.

WURTMAN, R.J., POHORECKY, L.A. & BALIGA, B.S. (1972). Adrenocortical control of the biosynthesis of epinephrine and proteins in the adrenal medulla. *Pharmac. Rev.*, **24**, 411–426.

(Received August 11, 1975. Revised November 3, 1975.)