*Br. J. Pharmac.* (1985), **85**, 249–253

# Comparison of the antagonistic effects of phentolamine on vasoconstrictor responses to exogenous and neurally released noradrenaline *in vivo*

C. Bell

Department of Physiology, University of Melbourne Medical Centre, Parkville, Victoria 3052, Australia.

**1** The antagonistic effects of the α-adrenoceptor blocking agent phentolamine on vasoconstrictor responses to intraluminal noradrenaline and lumbar sympathetic nerve stimulation were compared in the hindlimb of the anaesthetized dog.

**2** Sympathetic stimulation with 1 pulse or trains of 4–10 pulses at 0.4–40 Hz produced graded vasoconstrictor responses that were matched in amplitude by intra-arterial injections of $10^{-8}$–$10^{-6}$ g noradrenaline. Phentolamine (0.5 mg $kg^{-1}$ i.v.) attenuated amplitude-matched responses to both types of stimuli to quite similar extents.

**3** The extent of the effect of phentolamine on neurogenic responses was greater with 1 pulse stimulation than with trains, and greater with 4 pulse than with 10 pulse trains. The effect was maximal within 2 min of phentolamine administration and wore off in parallel with that on responses to injected noradrenaline.

**4** The results are consistent with the view that transmitter released from noradrenergic vasoconstrictor nerves acts primarily on subjunctional α-adrenoceptors.

## Introduction

It is generally recognized that the vasoconstriction produced by activation of noradrenergic vasomotor nerves is less affected by systemically administered α-adrenoceptor antagonists than is the vasoconstriction induced by direct intravascular introduction of noradrenaline (NA) (see, for instance, Dale, 1906; Nickerson, 1949; Laurence, 1973). This difference in susceptibility has been traditionally explained on the basis that the high concentration of noradrenaline produced in the subjunctional cleft following release from neuronal varicosities is sufficient to displace partially the antagonist from its binding sites on the subjunctional α-adrenoceptors (Ljung, 1969; Bevan & Su, 1971; Krnjević, 1974).

Recently, however, electrophysiological studies of transmission from vasomotor nerves to smooth muscle cells in isolated blood vessels have led to suggestions that the basis for resistance of neurogenic responses to α-adrenoceptor blockade lies in the existence of a specific population of subjunctional receptors for NA that are distinct from α-adrenoceptors (Hirst & Neild, 1980; Holman & Surprenant, 1980; Kuriyama & Makita, 1983), or in the fact that the primary neurotransmitter responsible for vascular muscle depolarization and constriction is not NA (Burnstock & Sneddon, 1984a,b).

Resolution of this situation clearly has important implications for the therapeutic manipulation of noradrenergic neurotransmission, but no data are available comparing the effects of α-adrenoceptor blockade on vasoconstriction induced *in vivo* by physiological patterns of sympathetic nerve stimulation and by intra-arterial NA. The present paper presents the results of such a study. Some of these results were communicated to the December, 1984 meeting of the Australian Society of Clinical and Experimental Pharmacologists (Bell, 1985).

## Methods

Adult mongrel dogs of either sex weighing 10–18 kg were anaesthetized with α-chloralose (70 mg $kg^{-1}$) following induction with sodium thiopentone, and artificially ventilated under positive pressure. Blood flow through the left femoral artery was recorded using an electromagnetic flow probe (Devices) and arterial blood pressure and heart rate were monitored from a branch of the right femoral artery. A fine (PE 50) polyethylene catheter was passed up another

branch of the right femoral artery to just above the aortic bifurcation, for the purpose of intra-arterial injection into the left femoral circulation. All parameters were recorded on a Grass 7B polygraph.

The left lumbar sympathetic chain was exposed through a flank incision between $L_4$ and $L_5$ ganglia, the chain was crushed rostrally, and the distal portion of the chain was stimulated with silver hook electrodes using single pulses and 4–10 pulse trains of 0.2 ms square wave pulses delivered at 0.4–40 Hz and supramaximal voltage (30 V) from a Grass S44 stimulator. The stimulation site was insulated with a pool of vaseline/paraffin wax, 1:1 (m.p. 43°C). All animals were treated with atropine methonitrate (0.4 mg kg$^{-1}$ i.v.) before commencement of the experiment in order to prevent hindlimb dilator responses to cholinergic sympathetic nerve activation. In some animals, sympathetic stimulation was accompanied by a minor degree of somatic activation in the hindlimb: in these cases pancuronium (Pavulon, Organon; 0.1 mg kg$^{-1}$ i.v.) was administered to prevent interference to blood flow responses by local functional hyperaemia. Adequacy of anaesthesia in the presence of pancuronium was monitored by the absence of pressor responses to mild peripheral noxious stimuli and by the absence of a corneal reflex during periodic withdrawal of pancuronium. In animals where visible somatic activation did not occur, no muscle relaxant treatment was given. There were no differences noted between the results obtained under these two conditions.

Other drugs used were noradrenaline bitartrate (Levophed, Winthrop) and phentolamine mesylate (Regitine, Ciba-Geigy).

Statistical analysis of the differences between means was performed using a two-tailed Student's *t* test.

## Results

For all 8 animals used, the mean arterial blood pressure (± s.e.mean) was 128 ± 6 mmHg, and the mean heart rate was 140 ± 10 beats min$^{-1}$. Left femoral blood flow was 108 ± 17 ml min$^{-1}$.

### *Responses to noradrenaline and sympathetic stimulation*

Intra-arterial injection of noradrenaline (NA) produced graded vasoconstrictor responses in the hindlimb over the dose range $10^{-8}$–$10^{-6}$ g. Sympathetic stimulation produced vasoconstrictor responses that were in the same range of magnitude as those produced by NA, the responses to single pulses being approximately equal to those to $5 \times 10^{-8}$ g NA, and those to trains of 4 or 10 pulses being approximately equal to responses to $2 \times 10^{-7}$ g and $6 \times 10^{-7}$ g NA,

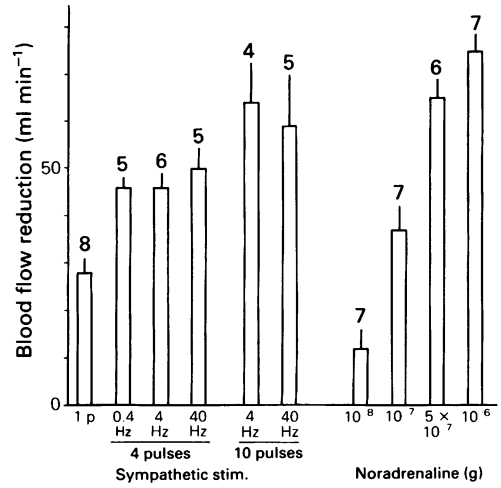


**Figure 1** Femoral vasoconstrictor responses (ml min$^{-1}$) to stimulation of the ipsilateral lumbar sympathetic trunk with single 0.2 ms pulses (1p) and with 4 pulse and 10 pulse trains, and to intra-arterial injection of noradrenaline in doses of $10^{-8}$–$10^{-6}$ g. The vertical lines represent one s.e.mean, and the numbers above each column indicate the number of experiments performed.

respectively (Figure 1). There was little difference between neurogenic responses to trains of the same number of pulses at different frequencies.

The vasoconstrictor responses to both NA and to sympathetic stimulation were accompanied by small rises in blood pressure (less than 20 mmHg) which were proportional in amplitude and similar in time course to the flow changes, and presumably reflected the increase in total peripheral resistance due to the femoral response. However, with neither form of stimulus was there a detectable reflex change in heart rate, and in the case of NA there was no indication of recirculation.

### *Effects of phentolamine on vasoconstrictor responses*

Slow intravenous injection of 0.5 mg kg$^{-1}$ phentolamine produced in most animals only transient changes in arterial blood pressure and femoral blood flow, although in two cases there was a sustained reduction of blood pressure by between 30 and 40 mmHg, and a concomitant sustained enhancement of resting femoral flow. In all experiments, phentolamine markedly attenuated responses to both NA and sympathetic stimulation within one minute of administration (Figures 2 and 3). In the case of NA, the effect represented a dose-ratio shift to the right of approximately 12. The extent of the effect was closely

similar for similarly sized responses to either NA or nerve stimulation, and the reduction of nerve-mediated responses was more pronounced with single pulses (87 ± 4%) than with trains (0.4 Hz, 69 ± 6%; 4 Hz, 71 ± 6%; 40 Hz, 67 ± 6%: $P < 0.05$ for single pulse vs 4 Hz). In the three experiments where both 4 pulse and 10 pulse trains of stimuli were used, the attenuation was consistently greater with the shorter train length. When data obtained in these three experiments with both 4 Hz and 40 Hz were pooled, the respective means were: 4 pulses 85 ± 3%; 10 pulses 66 ± 8% ($P < 0.05$). However, for any one train length there appeared to be no appreciable difference between the degrees of reduction caused by phentolamine at different frequencies (Figure 3).

Attenuation of responses to NA and to stimulation was maximal during the first few minutes after injection (Figure 2). Following this, there was progressive recovery of both types of response, which took place between 30–60 min in different animals. Although in general responses to nerve stimulation recovered fully before those to NA, during at least the first half of the recovery period both responses behaved in a closely parallel fashion (Figure 2).

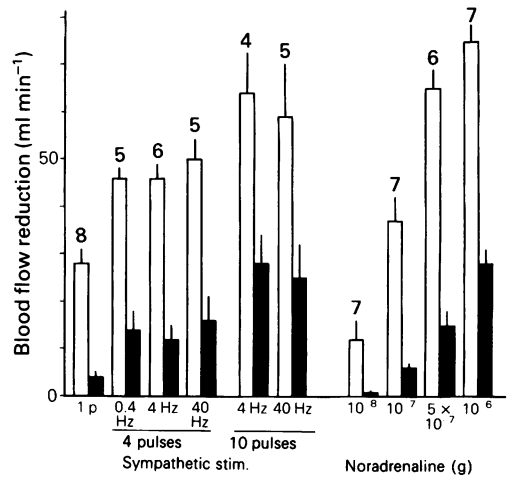


**Figure 3** Comparison of the extent of attenuation of the femoral vasoconstrictor responses shown in Figure 1 by phentolamine (0.5 mg kg$^{-1}$ i.v.). The open columns represent control values, and the filled columns those obtained after phentolamine. The vertical lines represent one s.e.mean, and the numbers above each pair of columns indicate the number of experiments performed.

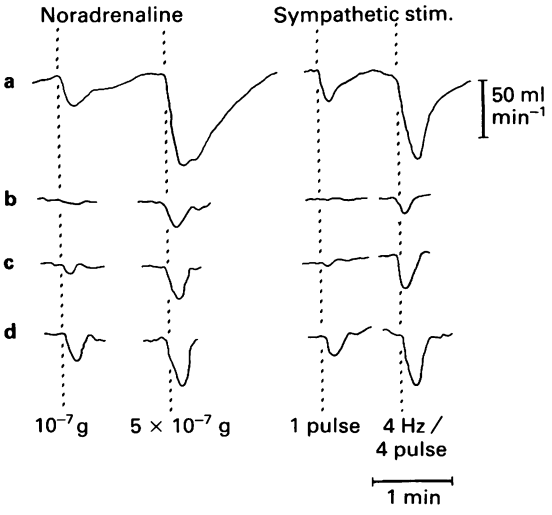


**Figure 2** Femoral vasoconstrictor responses to intra-arterial noradrenaline ($10^{-7}$ and $5 \times 10^{-7}$ g) and to lumbar sympathetic trunk stimulation (1 pulse and 4 pulses at 4Hz), (a) under control conditions, and (b) 2 min, (c) 10 min and (d) 20 min after administration of phentolamine 0.5 mg kg$^{-1}$ i.v. Resting femoral flows at each illustrated time were (a) 110, (b) 165, (c) 140 and (d) 130 ml min$^{-1}$.

## Discussion

Transmission from excitatory noradrenergic nerves to smooth muscle cells appears to have similar characteristics to those that underlie transmission at the somatic neuromuscular junction and at neuro-neuronal synapses (Burnstock & Holman, 1961; Holman & Hirst, 1977). Interaction of NA with subjunctional receptors produces excitatory junction potentials (e.j.ps) which depolarize the muscle cells. If this depolarization is sufficient, a muscle action potential is initiated and activates the contractile apparatus. On the basis of this sequence, therefore, alteration of the interaction of NA and the postjunctional receptors should be reflected in changes of amplitude or time course of the e.j.p., and concomitant changes in the amount of depolarization produced by a specified neural input.

Workers in a number of laboratories, using a variety of isolated vascular preparations from guinea-pig, rat and rabbit, have recently shown that the e.j.ps elicited by vasoconstrictor nerve stimulation are not affected by the α-adrenoceptor antagonists phentolamine and prazosin (Hirst & Neild, 1980; Holman & Surprenant, 1980; Cheung, 1982; Itoh *et al.*, 1983; Burnstock & Sneddon, 1984a,b). By contrast, depolarizing responses to applied NA are antagonized. Following repetitive stimulation in some preparations, a slow depolarization has been observed to follow the e.j.ps

(Cheung, 1982; Itoh *et al.*, 1983; Burnstock & Sneddon, 1984b). This response is also sensitive to blockade of α-adrenoceptors. These characteristics of the transmission process have been interpreted in two ways. Some workers have concluded that the subjunctional receptors that are the primary sites of interaction with neurogenic NA are of a type distinct from α-adrenoceptors (Hirst & Neild, 1980; Holman & Surprenant, 1980; Kuriyama & Makita, 1983). Alternatively, it has been suggested that the neurotransmitter that is responsible for e.j.p. production and action potential initiation is not NA (Burnstock & Sneddon, 1984a,b). The slow depolarization appearing during repetitive stimulation, which is sensitive to α-adrenoceptor blockade, is thought to be due to NA being released in larger quantities than occurs with single presynaptic impulses, and interacting with α-adrenoceptors outside the subjunctional area.

If either of these interpretations is the basis for the physiological mediation of neural vasoconstrictor activity, two predictions must be satisfied. Firstly, the effect of α-adrenoceptor antagonists on neurogenic constriction *in vivo* will be substantially less than that on responses to exogenous NA. Secondly, any attenuating effect of these blocking agents will be greater under conditions where the late, slow depolarization is most pronounced: thus neurogenic responses to single pulses will be affected less than those to impulse trains, and the effect will be progressively greater with longer trains.

The results of the present study demonstrate that, in the intact dog, neither of these predictions are upheld. When administered at a dose that is generally accepted to produce selective blockade of α-adrenoceptors, phentolamine produced attenuation of nerve-mediated and NA-induced vasoconstrictor responses to a similar extent. Furthermore, the effect on neural responses was considerably greater with single pulses than with trains of pulses, and was, if anything, less with longer trains of repetitive stimulation.

These data are entirely consistent with the view that the primary transmitter substance released by vasoconstrictor nerves in response to each action potential is NA, and that this interacts primarily with subjunctional α-adrenoceptors. The differences seen between the extent of antagonism of responses obtained using different numbers of pulses agree with what might be predicted to occur under conditions of competitive antagonism, as does the faster recovery of responses to nerve stimulation than that of responses to exogenous NA. However, the possibility exists that these phenomena may also involve the enhancement of transmitter release by phentolamine, through actions at prejunctional α-adrenoceptors (Starke, 1977) or other prejunctional sites (Bell, 1980). As the enhancement to transmitter release by α-adrenoceptor blocking agents appears to be greater under conditions of repetitive stimulation, it would be expected to interfere most with the postsynaptic action of phentolamine against longer trains of stimuli.

Despite the observed absence of effect of α-adrenoceptor antagonists on e.j.ps in isolated vessels, Holman & Surprenant (1980) and Hirst & Neild (1981) noted in the same experiments that high concentrations of these drugs attenuated mechanical neurogenic responses. This was explained on the basis of non-specific effects of the antagonists on the membrane properties of the smooth muscle cells, as the attenuation developed progressively during 20–30 min exposure of the tissue, whereas the specific antagonism of constrictor responses to exogenous NA was complete within seconds. In addition, in one of these studies responses to direct muscle stimulation were depressed (Holman & Surprenant, 1980). It should be noted that such non-specific actions cannot easily be implicated in the antagonism of neurogenic responses seen in the present study, as the effect of phentolamine was maximal immediately after administration, and responses to both nerve stimulation and to NA recovered over a similar period.

It is not clear whether the differences seen between the present results obtained *in vivo*, and those obtained in electrophysiological experiments with isolated vessels, reflect differences between the vasoconstrictor neuroeffector transmission processes in different species, or whether they imply a basic difference between the events that mediate functional vasoconstriction and those that are studied electrophysiologically. Whichever of these alternatives is correct, the results reinforce the difficulties of extrapolating from single cell studies to the whole organism.

I wish to thank Ciba-Geigy Australia Limited for a gift of Regitine and John Lynas for technical assistance.

## References

BELL, C. (1980). Effects of dopamine on adrenergic neuromuscular transmission in the guinea-pig vas deferens. *Br. J. Pharmac.*, **68**, 505–512.

BELL, C. (1985). Effect of phentolamine on noradrenergic vasoconstriction in the dog. *Clin. exp. Pharmac. Physiol.*, (in press).

BEVAN, J.A. & SU, C. (1971). Distribution theory of resistance of neurogenic vasoconstriction to alpha-receptor blockade in the rabbit. *Circulation Res.*, **28**, 179–187.

BURNSTOCK, G. & HOLMAN, M.E. (1961). The transmission of excitation from autonomic nerve to smooth muscle. *J. Physiol.*, **155**, 115–133.

BURNSTOCK, G. & SNEDDON, P. (1984a). Evidence that excitatory junction potentials in response to sympathetic nerve stimulation of some smooth muscles are mediated by ATP. *J. Physiol.*, **351**, 28P.

BURNSTOCK, G. & SNEDDON, P. (1984b). Electrical events underlying contractile responses to sympathetic nerve stimulation in rat tail artery. *J. Physiol.*, (in press).

CHEUNG, D.W. (1982). Two components in the cellular response of rat tail arteries to nerve stimulation. *J. Physiol.*, **328**, 461–468.

DALE, H.H. (1906). On some physiological actions of ergot. *J. Physiol.*, **34**, 163–206.

HIRST, G.D.S. & NEILD, T.O. (1980). Evidence for two populations of excitatory receptors for noradrenaline on arteriolar smooth muscle. *Nature*, **283**, 767–768.

HIRST, G.D.S. & NEILD, T.O. (1981). On the mechanism of action of prazosin at the sympathetic nerve-muscle junction of arterioles of the guinea-pig. *Br. J. Pharmac.*, **74**, 189P.

HOLMAN, M.E. & HIRST, G.D.S. (1977). Junctional transmission in smooth muscle and the autonomic nervous system. In *Handbook of Physiology* Section 1, Vol. 1, Part 1, ed. Kandel, E.R. pp. 417–462. Washington: American Physiological Society.

HOLMAN, M.E. & SURPRENANT, A.M. (1980). An electrophysiological analysis of the effects of noradrenaline and α-receptor antagonists on neuromuscular transmission in mammalian muscular arteries. *Br. J. Pharmac.*, **71**, 651–662.

ITOH, T., KITAMURA, K. & KURIYAMA, H. (1983). Roles of extrajunctional receptors in the response of guinea-pig mesenteric and rat tail arteries to adrenergic nerves. *J. Physiol.*, **345**, 409–422.

KRNJEVIĆ, K. (1974). Chemical nature of synaptic transmission in vertebrates. *Physiol. Rev.*, **54**, 418–540.

KURIYAMA, H. & MAKITA, Y. (1983). Modulation of noradrenergic transmission in the guinea-pig mesenteric artery: an electrophysiological study. *J. Physiol.*, **335**, 609–627.

LAURENCE, D.R. (1973). *Clinical Pharmacology*. 4th Edition, Edinburgh, London: Churchill.

LJUNG, B. (1969). Local transmitter concentrations in vascular smooth muscle during vasoconstrictor nerve activity. *Acta physiol. scand.*, **77**, 212–223.

NICKERSON, M. (1949). The pharmacology of adrenergic blockade. *Pharmac. Rev.*, **1**, 27–101.

STARKE, K. (1977). Regulation of noradrenaline release by presynaptic receptor systems. *Rev. Physiol. Biochem. Pharmac.*, **77**, 1–124.

*(Received October 26, 1984.*
*Accepted December 17, 1984.)*