Division of Biochemistry,
Central Drug Research Institute,
Lucknow, India and
Department of Physiology and Pharmacology,
University of North Dakota, School of Medicine,
Grand Forks, North Dakota 58201, USA.

RAMANUJ SEN
SURENDRA S. PARMAR*
S. R. GUHA**

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* Senior Foreign Visiting Scientist (National Science Foundation).
** Present Address: Indian Institute of Experimental Medicine, Calcutta, India.

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More on the blockade of neural and exogenous noradrenaline in vascular tissue

Recently published data from our laboratory do not support the traditional viewpoint on blockade of responses to exogenous and neural noradrenaline by α-adrenoceptor blocking agents (Wyse & Beck, 1972). It is generally accepted that responses to exogenous amine are more readily blocked by α-adrenoceptor blocking agents. Our data (Wyse & Beck, 1972) show that responses of small arteries from dog mesentery to exogenous and neural noradrenaline are blocked to an equivalent degree by low doses of phenoxybenzamine. Bevan, Su & Ljung (1973), have questioned our conclusion and in this paper we reply to the points they raised and present further data to reaffirm our original conclusion.

In vivo investigations by Levin & Beck (1967) and Miranda & Gomez (1970) have unequivocally demonstrated that sympathetic α-adrenoceptor responses of the hindlimb vasculature are more readily blocked by α-adrenoceptor blocking agents than are equivalent responses to injected amine. These findings were not noted by Bevan & others (1973) in their discussion of our data. Our *in vitro* experiments attempt to approximate to the *in vivo* situation by the use of small, densely innervated resistance blood vessels.

In the present experiments the methods were identical to those earlier outlined (Wyse & Beck, 1972) with the following differences. Experimental tissues were helical strips of rat ventral tail artery (0·7 to 0·9 mm o.d.). Square wave pulses of supramaximal current strength were delivered from a Grass Model S48 stimulator with a low output impedance (25 ohm). Both the "field" voltage (approximately 15 V) and the output current (calculated from voltage across a 1 ohm resistor) were monitored on an oscilloscope. Frequency-response curves were obtained by delivery of a 30 s train of pulses at selected frequencies with a 2 min pause between each stimulus during which the bath media were not changed. These parameters were chosen by experimentation as those giving maximal responses at each frequency without significant deterioration over several hours.

Final concentrations of noradrenaline are expressed as nmol (w/v) of the base litre^{-1}. Final concentrations of phenoxybenzamine (courtesy of H. A. Sheppard, Smith, Kline & French) are expressed as mol (w/v) of the hydrochloride salt litre^{-1}.

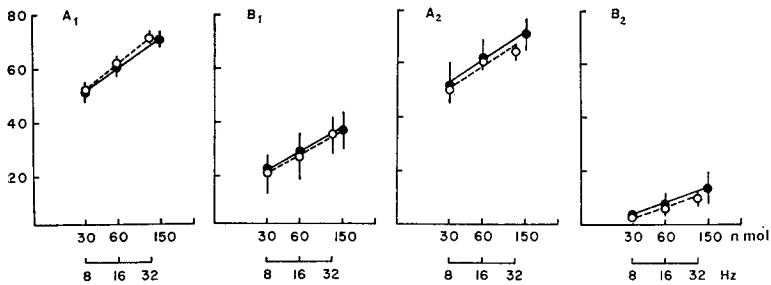

FIG. 1. Effect of phenoxybenzamine (A_1, B_1, $3{\cdot}3 \times 10^{-8}$; $A_2 B_2$ $1{\cdot}65 \times 10^{-7}$ mol) on responses to noradrenaline (closed circles, solid line) and transmural (open circles, broken line) stimulation. Pretreatment responses are shown in panel A and those after phenoxybenzamine in panel B. Each response is expressed as a % of the maximum response to noradrenaline. The upper abscissa is the noradrenaline concentration and the lower is the frequency of transmural stimulation Each point represents the mean of 9 (A_2, B_2) or 7 (A_1 B_1) experiments ± s.e.

Dose-response curves to noradrenaline and frequency-response curves to electrical stimulation were obtained in *each* strip. After several washes over 30 to 45 min, phenoxybenzamine was added to the baths and left for 10 min. Bath media were then rapidly changed six times to remove unbound phenoxybenzamine. After an additional 2 h during which bath media were changed every 15 min, the dose-response and frequency-response curves were repeated. Thus experiments were paired because neural and exogenous responses from the same strips were compared (the paired *t*-test was used and differences were considered significant when $P < 0{\cdot}05$). Responses were not retested until 2 h after exposure to phenoxybenzamine to ensure that stimuli were applied during the non-equilibrium phase of this drug's action.

Fig. 1 shows equivalent linear portions of the dose- and frequency-response curves before and after two concentrations of phenoxybenzamine. Both curves can be seen to remain parallel and to be shifted an exactly equivalent amount to the right by phenoxybenzamine. This is borne out by Table 1 which gives the paired *t*-test on responses to 8 Hz and 30 nmol of noradrenaline (approximate 'ED 50') before and after phenoxybenzamine. Equivalent blockade of both types of response by phenoxybenzamine ($3{\cdot}3 \times 10^{-8}$ and $1{\cdot}65 \times 10^{-7}$ mol litre^{-1}) has been a consistent finding in all our *in vitro* experiments with small arteries.

In the present experiments, and those previously reported (Wyse & Beck, 1972), the data were compared by the paired *t*-test and any differences were not statistically significant. Indeed, the extent of blockade was so nearly identical, that it seemed improbable that a biologically significant difference could be demonstrated irrespective of the number of experiments. Therefore, the suggestion of Bevan & others (1973) that our initial results support the "classical" view, has no substance in fact.

These authors also raised a question with respect to one of our parameters of electrical stimulation by suggesting that the stimulus voltage may have been submaximal. The voltage setting used initially (Wyse & Beck, 1972) was arrived at empirically and was determined to be supramaximal. The critical parameter with respect to supramaximal transmural stimulation is the amount of current delivered to the tissue. In the present experiments an additional circuit was included in the apparatus which permitted calculation of stimulus current. Thus it is possible to express the stimulus strength in terms of amperage rather than the nominal setting of the stimulator. The amount of current necessary to elicit a maximum response was about 400 mA. The present experiments were performed using a supramaximal stimulus current of 500 mA.

Table 1. *Paired t-test for responses (% of maximum) to 8 Hz and 30 nmol of noradrenaline (NA) before and after (A) 3·3 × 10⁻⁸ mol (B) 1·65 × 10⁻⁷ mol litre⁻¹ phenoxybenzamine (POB).*

	Before POB			After POB		
	8 Hz	30 nmol NA	Difference	8 Hz	30 nmol NA	Difference
A						
	47	52	+ 5	40	48	+ 8
	68	57	—11	7	6	— 1
	51	63	+12	5	28	+23
	48	62	+14	5	6	+ 1
	63	61	— 2	38	22	—16
	52	59	+ 7	29	32	+ 3
	60	51	— 9	56	50	— 6
	40	15	—25	3	0	— 3
	43	44	+ 1	2	6	+ 4
	Mean difference −0·9 s.e. ±4·1; *t* 0·21429; *P* <0·80.			Mean difference +1·4 s.e. ±3·5; *t* 0·40805; *P* <0·60.		
B						
	59	67	+ 8	5	1	— 4
	47	43	— 4	8	3	— 5
	60	85	+25	3	13	+10
	62	56	— 6	2	12	+10
	47	53	+ 6	0	0	0
	28	27	— 1	0	0	0
	46	30	—16	3	1	— 2
	Mean difference +1·7 s.e. ±4·9; *t* 0·34899; *P* <0·70.			Mean difference +1·3 s.e. ±2·4; *t* 0·53854; *P* <0·60.		

Bevan & others (1973) correctly pointed out that phenoxybenzamine is known to block re-uptake of noradrenaline. They also suggest that blockade of re-uptake would result in a rapid fall off of transmitter release and cited Su & Bevan (1970) to support the suggestion. But that work used a superfused preparation quite different from the preparation we used. Superfused strips would be more susceptible to transmitter depletion when the re-uptake mechanism is somewhat inhibited. The important point is that the dose of phenoxybenzamine in the experiments of Su & Bevan (1970), and that used by most others (e.g. Bell & Vogt, 1971) to block noradrenaline re-uptake, is 200 to 6000 times the concentrations we used. Phenoxybenzamine concentration is a crucial point with regard to blockade of amine re-uptake versus blockade of α-adrenoceptors. Low doses of this drug, which cause almost 100% blockade of adrenoceptor responses, only moderately increase transmitter output (Häggendal, Johansson & others, 1972). Therefore it should be possible to achieve partial blockade of adrenoceptor responses with doses of phenoxybenzamine that have little or no effect on re-uptake. We used low doses in our work to avoid the complications introduced when amine re-uptake is blocked. Objective evidence that our aim was achieved is provided by the data. If re-uptake were blocked and there were a rapid fall off of transmitter release, responses elicited later during the frequency-response curve (higher frequencies) would be reduced proportionally more than responses elicited earlier (lower frequencies), because transmitter release would become progressively less with each stimulus. Under these circumstances the frequency-response and noradrenaline dose-response curves, which are initially parallel, would not be parallel after phenoxybenzamine; the slope of the frequency-response curve would be reduced. This clearly does not happen (Fig. 1). Thus there is no reason to believe that reduction of the neural responses is due to any mechanism other than blockade of α-adrenoceptors.

From the reasoning of Bevan & others (1973), it follows that higher doses of

phenoxybenzamine are much more likely to block noradrenaline re-uptake and to result in decreased transmitter release. Yet in spite of using a concentration of phenoxybenzamine which was 20 times our highest dose, Bevan & Su (1971) reported a *lesser* rather than an equal or a greater blockade of neural responses. Stimulation only once at one frequency after phenoxybenzamine would not guarantee that transmitter release was undiminished. In our studies, it was sometimes difficult to eliminate all responses to electrical stimulation after phenoxybenzamine. This may suggest either: (*a*) a few receptors are not normally accessible to phenoxybenzamine; or, (*b*) electrical stimulation may cause a small contraction of smooth muscle fibres which is unrelated to release of noradrenaline. In either case, electrical responses would be resistant to blockade by phenoxybenzamine.

The present experiments reaffirm and extend our initial conclusion (Wyse & Beck, 1972). In small arteries studied *in vitro* using transmural stimulation, responses to neural and exogenous noradrenaline are blocked to an equivalent degree by low doses of phenoxybenzamine. Obviously we do not agree that the points raised by Bevan & others (1973) "seriously weakened" our conclusion.

With regard to their discussion of the "distribution theory", it is our view that "temporal distribution" of neurotransmitter or "intimate relationship" of nerve ending and effector cell cannot be important considerations for a long lasting non-equilibrium α-adrenoceptor antagonist such as phenoxybenzamine.

It seems clear that further experimentation is necessary to explain fully the differences in results observed between *in vivo* and *in vitro* experiments. Our *in vitro* data have partially resolved the discrepancy. They demonstrate that blockade of neural and exogenous responses of arteries near in size to resistance vessels of the hindlimb vasculature is quantitatively different from that reported for the large blood vessels commonly used *in vitro*. However, they do not satisfactorily explain observations, made by Levin & Beck (1967) and confirmed by Miranda & Gomez (1970), which unequivocally demonstrate that the neural response is more effectively blocked by phenoxybenzamine in the intact preparation. Neither do the publications of Bevan and his colleagues.

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Division of Pharmacology and Therapeutics,
Faculty of Medicine,
The University of Calgary,
Calgary, Alberta, Canada, T2N 1N4

D. GEORGE WYSE*
LLOYD BECK**

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* Medical Scientist Fellow of the Canadian Heart Foundation
** President address: Department of Pharmacology, School of Medicine, The University of Minnesota, Duluth, Minnestoa, U.S.A.

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