Br. J. Pharmac. (1979), **67**, 401–407

AN EXAMINATION OF THE NEGATIVE FEEDBACK FUNCTION OF PRESYNAPTIC ADRENOCEPTORS IN A VASCULAR TISSUE

CHI-CHUNG CHAN & STANLEY KALSNER

Department of Pharmacology, School of Medicine, University of Ottawa, Ottawa, Ontario, Canada

1 The hypothesis was examined that presynaptic α-adrenoceptors exert a negative feedback function regulating noradrenergic transmission.

2 Renal artery strips from cattle, pre-incubated with [^{3}H]-noradrenaline, were stimulated with 300 pulses at 5 different frequencies, spanning the physiological range, and the efflux of tritium assessed both in the absence and presence of functional presynaptic receptors.

3 Considerable variation in the synaptic level of free and active noradrenaline with increasing frequency was apparent from the rates of development and the magnitudes of the mechanical responses but the overflow of tritium was constant at 1, 2, 10 and 15 Hz and slightly elevated at 5 Hz, providing no evidence for presynaptic modulation of release.

4 Phenoxybenzamine (3.3×10^{-5} M) enhanced the overflow of tritium most at the lowest frequency tested and to a similar extent at the other test frequencies, except 10 Hz where its effect was slightly reduced.

5 The conditions of the present experiments appeared optimal for the operation of the negative feedback system and the failure to observe an increased effectiveness of the antagonist with increasing frequency indicates that the physiological relevance of such a system is highly questionable and suggests that it may not function at all.

Introduction

Proponents of the presynaptic receptor hypothesis assert that sympathetic nerve terminals possess a negative feedback system comprising adrenoceptors of the α-type which are activated by endogenously released transmitter leading to a reduction of subsequent noradrenaline release (see reviews by Rand, McCulloch & Story, 1975; Langer, 1977; Starke, 1977). The only clear support for the operation of such an auto-inhibitory feedback loop under ordinary conditions of nerve transmission is the often confirmed finding that phenoxybenzamine and certain other α-adrenoceptor antagonists increase the stimulation-induced efflux of [^{3}H]-noradrenaline, presumably reflecting blockade of the critical presynaptic sites of noradrenaline action. However, it was recently reported that phenoxybenzamine enhanced the overflow of tritiated noradrenaline and the mechanical contraction in guinea-pig vasa deferentia in response to a single brief (1 ms) pulse administered by field stimulation (Kalsner, 1979). This experimental condition precluded the involvement of presynaptic receptors since noradrenaline released by a single pulse cannot retroactively modulate its own release. Such a challenge to the currently accepted interpretation of the mechanism of action of phenoxybenzamine requires confirmation by means of other experimental protocols and it provided the incentive for the present investigation.

The experiments described here utilized a renal artery preparation preincubated with [^{3}H]-noradrenaline to assess if blockade of a negative-feedback loop adequately explains the enhancing effect of the haloalkylamine antagonist on transmitter efflux. Although phenoxybenzamine increased consistently the efflux of tritium, the relationship between stimulation frequency and antagonist effectiveness was not at all in accord with the requirements of a negative feedback system regulating neurotransmitter release.

Methods

Tissue preparation

Renal arteries from cattle were dissected out at the slaughter house and transported promptly to the

0007-1188/79/110401-07 $01.00

laboratory in cold previously oxygenated Krebs-Henseleit solution. After removal of visible fat and connective tissue, the arteries were cut into spiral strips of 4 × 30 mm, weighing from 250 to 350 mg. The strips were incubated for 60 min in 4.0 ml of oxygenated (5% CO_2 in O_2) Krebs-Henseleit (Krebs) solution (NaCl 115.3, KCl 4.6, $CaCl_2$ 2.3, $MgSO_4$ 1.1, $NaHCO_3$ 22.1, KH_2PO_4 1.1, glucose 7.8 and disodium ethylene diamine tetra-acetic acid 0.03 mM) containing (−)-[7-^{3}H]-noradrenaline (10 μCi/ml, 6.7×10^{-7} M) then washed with fresh Krebs solution. The strips were mounted under 4 g tension and mechanical responses were recorded isometrically by means of force-displacement transducers and a Grass polygraph. Preparations were superfused continuously with warmed (37°C) and oxygenated Krebs solution by a gravity-feed set-up at a constant pressure of 60 mmHg and a flow rate of 4 ml/min.

Stimulation parameters

The strips were suspended between platinum wire electrodes fixed vertically on opposite sides of the strips and they were stimulated transmurally. A fixed number (300) of biphasic pulses of 1.0 ms duration and at supramaximal voltage was delivered to the vessels at either 1, 2, 5, 10 or 15 Hz with Grass model S6 stimulators.

Drugs and radiochemicals

The drugs used and their sources were: cocaine hydrochloride (May & Baker Ltd.), normetanephrine hydrochloride (Calbiochem), phenoxybenzamine hydrochloride (Smith, Kline & French Canada Ltd.). The radioisotope, (−)-[7-^{3}H]-noradrenaline hydrochloride (specific activity 15 Ci/mmol) was obtained from the Radiochemical Centre, Amersham. It was diluted to a stock concentration of 100 μCi/ml (6.7×10^{-6} M) in ascorbic acid (50 μg/ml) and stored at 4°C in 10 ml aliquots under nitrogen gas. To obtain a final concentration of 10 μCi/ml (6.7×10^{-7} M) in the incubation medium, 0.4 ml of this stock solution was added to 3.6 ml of Krebs solution. The cocaine (3 μg/ml; 8.8×10^{-6} M), normetanephrine (2.2 μg/ml; 1×10^{-5} M) and phenoxybenzamine (10 μg/ml; 3.3×10^{-5} M) were dissolved directly in the Krebs solution.

Protocols

After a 120 min equilibration period, each strip was stimulated at the desired frequency, followed after 14 min by another period of stimulation at a second test frequency. No more than three frequencies were used on any given strip and they were given in random sequence. Experiments were always done on four strips at a time, all taken from the same artery. Two strips always received phenoxybenzamine after intitial testing and two served as controls; 30 min after the onset of exposure to the antagonist and in its presence, a second run of stimulations identical to the first was performed on all treated and control strips.

Efflux of [^{3}H]-noradrenaline

The efflux of [^{3}H]-noradrenaline from the preparations was determined by counting 1.0 ml aliquots of the 16.0 ml superfusate collected in vials by a fraction collector which rotated once every 4 min. The aliquots were then transferred to vials containing 10 ml of Aqueous Counting Scintillant (Amersham) and counted to a 1% error in a Beckman LS-150 counter with automatic external standardization to determine efficiency.

Basal efflux is expressed as disintegrations per minute (d/min) and referred to as the total radioactivity detected in the 4 min sample collected immediately before stimulation. Stimulation-induced efflux was calculated as the difference between basal efflux and the total d/min detected in the 4 min samples collected during and immediately after stimulation. Transmural stimulation was always begun at the start of a 4 min collection period.

Mean data on efflux and mechanical response are presented with their standard errors and Student's paired t test was used for all intrastrip comparisons with the unpaired test used for comparisons between groups; P values of less than 0.05 were considered significant.

Results

Efflux of [^{3}H]-noradrenaline and responses

The renal artery preparation of cattle responded to field stimulation with 300 pulses at 1, 2, 5, 10 and 15 Hz with effluxes of tritium elevated substantially above basal levels and with definite increases in muscle tension. In all experiments cocaine (8.8×10^{-6} M) and normetanephrine (1×10^{-5} M) were routinely present in the Krebs solution superfusing the vascular strips to block neuronal and extraneuronal routes of noradrenaline inactivation. As shown in Figure 1 the mean stimulation-induced efflux in initial control tests with all strips was constant, regardless of the frequency at which the 300 pulses was delivered to the tissue, except at 5 Hz where a somewhat higher output was recorded. In fact, the minimum efflux values (lower limit of the 95% confidence interval) at the two highest frequencies fall within the 95% confidence intervals for efflux at the two lowest frequencies. The mechanical responses, however, varied pre-

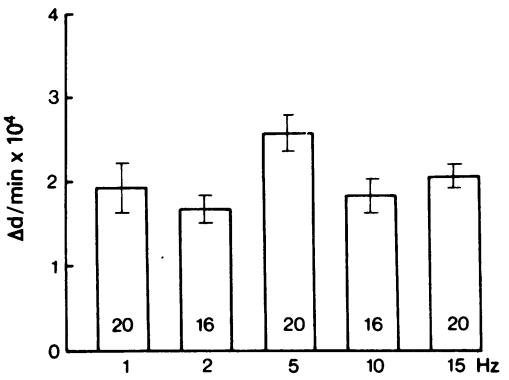

Figure 1 Relationship between stimulus frequency and overflow of tritium in cattle renal artery. Stimulation was with 300 pulses at each test frequency. Number of values in each group are shown within columns. Mean value at 5 Hz differed from that at 2 Hz ($P < 0.01$) and from that at 10 Hz ($P < 0.05$). All other values did not differ significantly from each other using t test for unpaired data. The means and 95% confidence limits for efflux at each of the indicated frequencies from 1 to 15 Hz are 1.93 ± 0.65, 1.67 ± 0.34, 2.58 ± 0.48, 1.83 ± 0.40 and $2.06 \pm 0.29 \times 10^4$ d/min, respectively.

dictably with the frequency of stimulation reflecting the interval between pulses, the total duration of stimulation and consequently the peak synaptic accumulation of transmitter.

The peak vascular responses of the same strips whose overflow of tritium is described in Figure 1, increased with increasing frequency in the absence of phenoxybenzamine (Figure 2). Although stimulation at 1 and 2 Hz allowed sufficient time (5 and 2.5 min respectively) for the responses to plateau, the briefer and more concentrated expulsion of the same total amount of transmitter at the higher frequencies yielded responses which developed more rapidly, were of more intense magnitudes but curtailed abruptly by the termination of stimulation. The mean times taken for responses to peak were 115.9 ± 15.7, 97.6 ± 7.2, 62.4 ± 1.7, 44.1 ± 2.9 and 44.1 ± 3.7 s as the frequency increased from 1 to 15 Hz. Confirmation that the observed responses resulted from activation of sympathetic nerve terminals was obtained with guanethidine. A 30 min exposure of renal artery strips to this neuronal blocking agent eliminated or reduced all observable contractile responses to field stimulation; that to the highest frequency (15 Hz) was reduced to only 22.4% of control values.

Effects of phenoxybenzamine on tritium efflux

In preparations not treated with the adrenoceptor antagonist, the efflux of tritium did not differ substantially when a second period of stimulation, at any of the given test frequencies, followed the first after a 30 min interval (Table 1). In matching strips, taken from the same renal arteries, and exposed to phenoxybenzamine in the interval between periods of stimu-

Table 1 The ratios of transmitter overflow in the second period of stimulation and the first period in the absence and presence of phenoxybenzamine (Pbz)

Experimental group	*Stimulation frequency* (Hz)	*No. of values*	*Transmitter overflow d/min* ($\times 10^4$) *1st run*	*2nd run*	*Overflow ratio 2nd run/1st run*
Control	1	10	1.91 ± 0.57	1.44 ± 0.20	(a) 1.05 ± 0.16
Pbz	1	10	1.94 ± 0.27	5.25 ± 0.82	(b) $2.88 \pm 0.25^{*}_{**}$
Control	2	8	1.58 ± 0.31	1.45 ± 0.26	(c) 0.95 ± 0.05
Pbz	2	8	1.76 ± 0.12	3.41 ± 0.25	(d) $1.98 \pm 0.17^{*}$
Control	5	10	2.34 ± 0.30	2.11 ± 0.23	(e) 0.94 ± 0.07
Pbz	5	10	2.81 ± 0.30	5.33 ± 0.48	(f) $2.01 \pm 0.16^{*}$
Control	10	8	1.76 ± 0.32	1.66 ± 0.36	(g) 0.93 ± 0.06
Pbz	10	8	1.90 ± 0.23	2.69 ± 0.40	(h) $1.43 \pm 0.10^{*}_{***}$
Control	15	10	1.99 ± 0.20	1.70 ± 0.13	(i) 0.89 ± 0.06
Pbz	15	10	2.13 ± 0.19	3.70 ± 0.62	(j) $1.76 \pm 0.24^{*}$

Phenoxybenzamine, when given, was administered in the interval between runs, as described in Methods. * Indicates ratios of treated groups significantly different from ratios of corresponding control groups with a $P < 0.01$. ** Indicates group significantly different from groups (d), (f), (h), (j) with a $P < 0.02$. *** Indicates group significantly different from groups (d) and (f) with a $P < 0.02$. The ratios of all other treated groups do not differ significantly from each other. Untreated groups do not differ significantly from each other. The correlation coefficient (r) between length of stimulus interval and ratio of transmitter overflow in the presence of phenoxybenzamine was 0.929 with a $P < 0.01$.

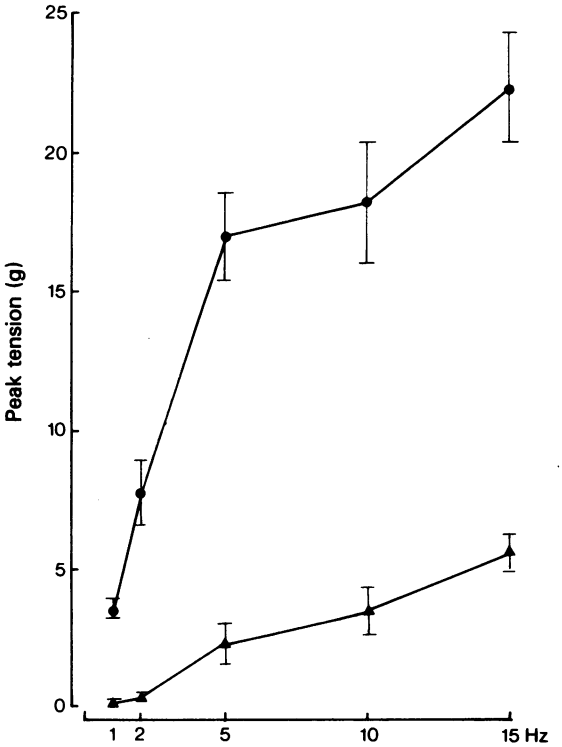

Figure 2 Frequency-response curves in renal artery of cattle in the absence and presence of phenoxybenzamine (3.3×10^{-5} M). Stimulation was with 300 pulses at each test frequency. Mean values shown are those for initial control tests (●) in all strips whose tritium overflow values are presented in Figure 1 and also those obtained during the second run in all strips pretreated with phenoxybenzamine in the interval between runs (▲). Second run values in untreated strips (not shown) did not differ significantly from those of first runs.

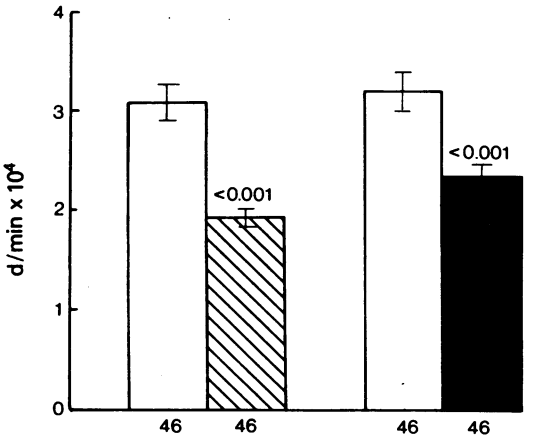

Figure 3 Spontaneous efflux of tritium in renal arteries pretreated with cocaine (8.8×10^{-6} M) and normetanephrine (1×10^{-5} M). Basal efflux in two sets of arteries obtained prior to each frequency test during first run of nerve-induced stimulations (open columns) and during second run in the absence (hatched column) and presence (filled column) of phenoxybenzamine (3.3×10^{-5} M). Number of values in each group are shown below columns. Probability comparisons, by the paired *t* test are between first and second runs in each group.

lation, the overflow of tritium was significantly increased at all five of the test frequencies, as assessed by an intrastrip comparison of the ratio of efflux in second versus first periods of stimulation. The effectiveness of phenoxybenzamine was greatest at the lowest frequency of 1 Hz and diminished slightly but significantly at the higher frequencies. The efflux ratios at 2, 5, 10 and 15 Hz did not differ significantly from each other with one exception at 10 Hz, where the effect of the antagonist although not significantly different from that at 15 Hz was significantly but modestly less than at 2 and 5 Hz.

Effect of phenoxybenzamine on mechanical response

The α-andrenoceptor antagonist profoundly depressed the responses to nerve stimulation at all test frequencies. As shown in Figure 2 the frequency-response curve was shifted to the right and its maximum decreased. These effects are characteristic of a non-competitive antagonism due to the formation of a covalent bond through alkylation with some component of the α-site (Nickerson & Collier, 1975).

Spontaneous efflux of tritium

The basal efflux of tritium, measured immediately before each stimulation, decreased significantly between first and second runs in untreated strips (Figure 3). In preparations exposed to phenoxybenzamine between runs, a decrease was also apparent but an intrastrip comparison of ratios revealed that it was significantly less ($P < 0.001$) than in control strips. The ratios were 0.64 ± 0.01 and 0.76 ± 0.02 in control and treated strips, respectively.

Discussion

Inhibition of transmitter release by previously released noradrenaline is assumed to 'play a major physiological role in noradrenergic transmission' (Langer, 1977; see also reviews by Rand *et al.*, 1975; Starke, 1977). This negative feedback function of presynaptic adrenoceptors located on sympathetic nerve terminals although attractive is an unsubstantiated hypothesis. The notion that presynaptic α-adrenocep-

tor sites are routinely activated during sympathetic nerve excitation, once threshold is reached, in proportion to the concentration of transmitter achieved in the synaptic region, rests entirely on the experimental findings that noradrenaline and other adrenoceptor agonists inhibit release while phenoxybenzamine and some other α-antagonists block this effect and themselves enhance release (Haggendal, 1970; Farnebo & Hamberger, 1970; 1971; Langer, 1970; Kirpekar & Puig, 1971; Starke, Montel & Schümann, 1971; McCulloch, Rand & Story, 1972; Starke, Endo & Taube, 1975).

The purpose of the present investigation was to examine the relationship between frequency of stimulation and transmitter efflux in the absence and presence of functional presynaptic receptors. Only in this way can the purported negative feedback function of these phenoxybenzamine-sensitive sites be appropriately assessed. The renal artery of cattle, responds as a characteristic vascular preparation with sympathetic nerve activation eliciting tension increases over the broad range of stimulation frequency employed. As emphasized by Folkow & Neil (1971) the physiological range of discharge in tonically active vasoconstrictor fibres is narrow, with maximal effector responses usually achieved below 10 Hz. This is supported here by the finding that the contractile tension changes varied greatly between 1 and 5 Hz and less so at the highest frequences. However, it should be noted that stimulation at the higher frequencies intentionally was not maintained sufficiently long to achieve plateau responses, so that a fixed number of pulses could be delivered to the tissue at all five test frequencies. This was desirable to allow comparisons in efflux to be made between frequencies without the introduction of compounding variables associated with train length. Thus, the interval between stimuli, in the present experiments varied between 66 and 999 ms, allowing variations in the peak synaptic accumulation of transmitter probably as extreme as any likely to be encountered under physiological conditions. Evidence that striking differences in synaptic levels of free and active transmitter were indeed achieved is readily provided by an examination of the already described mechanical responses. The rates of contraction and the magnitudes of these responses reflect the temporal and spatial dynamics of the released noradrenaline.

It was found that the efflux of [^{3}H]-noradrenaline was essentially unaffected by the frequency of stimulation with similar amounts being released by 300 pulses at all test frequencies except for 5 Hz. Thus, by examination of control data alone, no evidence for the operation of a negative feedback system was obtained; that is the expected decrease in transmitter output per pulse as the frequency increases with a fixed number of pulses. In the nictitating membrane of cat and mouse vas deferens the outflow per pulse is also relatively constant over a broad frequency range (Farnebo & Malmfors, 1971; Henderson, Hughes & Kosterlitz, 1975), although the number of pulses was not kept constant in the latter study. However, Rand, Story, Allen, Glover & McCulloch (1973) observed that stimulation-induced efflux in the rabbit ear artery rose to a peak at 5 Hz and declined with higher frequency (variable pulse number) and Hughes (1972) found in rabbit portal vein and vas deferens that per pulse output of transmitter increased in the range of 2 to 15 Hz with a fixed pulse number, representing a facilitation of transmitter output with increasing frequency. Thus no single pattern applicable to all effectors is evident. Since it could be argued that a proportional facilitation of transmitter release with increasing frequency and of unknown mechanism obscured the depression of release due to presynaptic receptor activation, the tissues were routinely treated with phenoxybenzamine to assess precisely the contribution of the presynaptic system at each of the test frequencies.

As expected, and in confirmation of numerous other studies the haloalkylamine increased the efflux of tritium, even in the presence of inhibitors of neuronal and extraneuronal uptake. However, no support whatsoever for the operation of a negative feedback system was obtained. Since phenoxybenzamine supposedly eliminated the restraint on release imposed by presynaptic α-receptor activation the stimulation-induced efflux in its presence should be greater than in its absence and it should be so in proportion to the degree of functional autoinhibition operative at each specific frequency. According to theory, the difference in overflow in the presence and absence of inhibitor should be greater the higher the frequency, until conditions of stimulation are employed which fully saturate the system. This was not observed. The effect of the antagonist was positively correlated, instead, with the length of the interval between stimuli.

The concentration of phenoxybenzamine used in this study was intentionally high, in the range reported by others to produce a maximal blockade of the presynaptic system as evidenced by efflux (Hughes, 1972; Rand *et al.*, 1975; Henderson *et al.*, 1975). In addition, a 30 min contact time prior to the onset of stimulation was employed. Phenoxybenzamine produces an irreversible, non-equilibrium or non-competitive blockade of adrenoceptors through the formation of a reactive intermediate characteristic of haloalkylamines (Nickerson & Collier, 1975). Even after washout of the antagonist, presynaptic receptor blockade in vascular tissue, as assessed by transmitter overflow, is maintained over a period of 6 to 8 h with only a 10 to 15% decline in transmitter output (Hughes, 1972). Starke (1972) has shown that 0.1 μg/ml of exogenous noradrenaline cannot break

through a blockade by phenoxybenzamine, even when the antagonist is used at a concentration ten times below that employed here and with a contact time of only 16 min before testing. It appears that any objection to the present data on the grounds that transmitter breaks through the blockade in such a way as to nullify precisely the visibility of a negative feedback function is unsupportable.

Another possible objection to the present data is that the presynaptic system is already fully saturated with noradrenaline, and operating maximally, even at the lowest frequency here employed. If such were the case then it can be obviously concluded on that basis alone that the system has no relevence to physiological parameters of stimulation and makes no serious contribution to the regulation of transmitter release under ordinary conditions of nerve activity.

Surprisingly little experimental support for a negative feedback function of presynaptic receptors is available in the literature. In most cases, the effect of phenoxybenzamine has been examined only at one moderate and one unphysiologically high frequency but with consistent results which offer no substantiation for a system sensing the biophase level of free and active noradrenaline. Bell & Vogt (1971) observed that phenoxybenzamine enhanced output in a guinea-pig artery at 5 Hz but not at 25 Hz and a similar relationship was reported with 2 and 50 Hz in rat vas deferens (Vizi, Somogyi, Hadhazy & Knoll, 1973) and with 5 and 30 Hz in cat spleen (Langer, Dubocovich & Celuch, 1975). Also, Brown & Gillespie (1957) and Kirpekar & Cervoni (1963) found that haloalkylamine antagonists enhanced output at 10 but not at 30 Hz in cat spleen. Langer (1977) commented that 'it is likely that at high frequencies of nerve stimulation the negative feedback regulatory mechanism which is mediated by presynaptic α-receptors does not play an important role in the regulation of transmitter release'. The critical concern then becomes, when does it play an important role?

Those few experiments which examine the relative effectiveness of the antagonist under more moderate conditions do not show an increasing effect with increasing frequency. Hughes (1972), who at the time attributed the effect of phenoxybenzamine on transmitter efflux entirely to blockade of inactivation pathways, observed in the rabbit vas deferens that, in the presence of cocaine, the haloakylamine increased output to a similar extent when 240 pulses were given at either 2, 6 or 16 Hz. McCulloch, Bevan & Su (1975) reported that phenoxybenzamine increased output to the same extent at 4 and 8 Hz in rabbit pulmonary artery (with inactivation pathways left intact) but attributed this to an inadequate exposure time with a moderate concentration of antagonist.

The only evidence which seems to reinforce the claim of a feedback function for released noradrenaline is that of Enero & Langer (1973) and Cubbedu & Weiner (1975) which shows that depletion of transmitter with either reserpine or α-methyl-*p*-tyrosine reduces the effectiveness of phenoxybenzamine in enhancing outlow. This has been interpreted as the consequence of a decreased amount of transmitter released into the synaptic space with each impulse. However, it is obvious, that until the mechanism of phenoxybenzamine action is established its actual mode of interaction with noradrenaline depleting agents remains conjecture and such data can provide only highly indirect support for the routine operation of a negative feedback system on sympathetic nerve terminals.

From the present data a picture emerges of a sensing system mediating inhibition of output which, if it exists at all, responds with a relatively fixed signal regardless of the biophase concentration of agonist and then, according to the available evidence, becomes exhausted at very high frequencies. Thus, no apparent physiological regulatory function can be assigned to it. Further, a reservation must be expressed, particularly since the recent finding that phenoxybenzamine elevates above control values the efflux of tritium and the mechanical response when only a single pulse is delivered to the guinea-pig vas deferens (Kalsner, 1979), that the hypothesis of specific functional presynaptic adrenoceptors itself has been prematurely acknowledged and that the mechanism of phenoxybenzamine action must be sought elsewhere.

We thank Mr Mohammad Suleiman and Mr Robert Dobson for expert technical assistance. This research was supported by grants from the Ontario Heart Foundation and the Medical Research Council.

References

BELL, C. & VOGT, M. (1971). Release of endogenous noradrenaline from an isolated muscular artery. *J. Physiol.*, **215**, 509–520.

BROWN, G.L. & GILLESPIE, J.S. (1957). The output of sympathetic transmitter from the spleen of the cat. *J. Physiol.*, **138**, 81–102.

CUBEDDU, L.X. & WEINER, N. (1975). Nerve-stimulation mediated overflow of norepinephrine and dopamine-β-hydroxylase. III. Effects of norepinephrine depletion on the alpha presynaptic regulation of release. *J. Pharmac. exp. Ther.*, **192**, 1–14.

ENERO, M.A. & LANGER, S.Z. (1973). Influence of reserpine-

induced depletion of noaradrenaline on the negative feed-back mechanism for transmitter release during nerve stimulation. *Br. J. Pharmac.*, **49**, 214–225.

FARNEBO, L.-O. & HAMBERGER, B. (1970). Effects of desipramine, phentolamine and phenoxybenzamine on the release of noradrenaline from isolated tissues. *J. Pharm. Pharmac.*, **22**, 855–856.

FARNEBO, L.-O. & HAMBERGER, B. (1971). Drug-induced changes in the release of [^{3}H]-noradrenaline from field stimulated rat iris. *Br. J. Pharmac.*, **43**, 97–106.

FARNEBO, L.-O. & MALMFORS, T. (1971). ^{3}H–noradrenaline release and mechanical response in the field stimulated mouse vas deferens. *Acta physiol. scand.*, Suppl., **371**, 1–18.

FOLKOW, B. & NEIL, E. (1971). *Circulation*. New York: Oxford University Press.

HAGGENDAL, J. (1970). Some further aspects on the release of the adrenergic transmitter. In *New Aspects of Storage and Release Mechanisms of Catecholamines*. ed Schümann, H. J. & Kronenberg, G. p. 100. Berlin: Springer-Verlag.

HENDERSON, G., HUGHES, J. & KOSTERLITZ, H.W. (1975). The effects of morphine on the release of noradrenaline from the cat isolated nictitating membrane and the guinea-pig ileum myenteric plexus-longitudinal muscle preparation. *Br. J. Pharmac.*, **53**, 505–512.

HUGHES, J. (1972). Evaluation of mechanisms controlling the release and inactivation of the adrenergic transmitter in the rabbit portal vein and vas deferens. *Br. J. Pharmac.*, **44**, 472–491.

KALSNER, S. (1979). Single pulse stimulation of guinea-pig vas deferens and the presynaptic receptor hypothesis. *Br. J. Pharmac.*, **66**, 343–349.

KIRPEKAR, S.M. & CERVONI, P. (1963). Effect of cocaine, phenoxybenzamine and phentolamine on the catecholamine output from spleen and adrenal medulla. *J. Pharmac. exp. Ther.*, **142**, 59–70.

KIRPEKAR, S.M. & PUIG, M. (1971). Effect of flow-stop on noradrenaline release from normal spleens and spleens treated with cocaine, phentolamine or phenoxybenzamine. *Br. J. Pharmac.*, **43**, 359–369.

LANGER, S.Z. (1970). The metabolism of [^{3}H]-noradrenaline released by electrical stimulation from the isolated nictitating membrane of the cat and from the vas deferens of the rat. *J. Physiol.*, **208**, 515–546.

LANGER, S.Z. (1977). Presynaptic receptors and their role in the regulation of transmitter release. *Br. J. Pharmac.*, **60**, 481–497.

LANGER, S.Z., DUBOCOVICH, M.L. & CELUCH, S.M. (1975). Prejunctional regulatory mechanisms for noradrenaline release elicited by nerve stimulation. In *Chemical Tools in Catecholamine Research*, Vol. II. p. 183. ed. Almgren, O., Carlsson, A. & Engel, J. Amsterdam: North-Holland Publishing Company.

MCCULLOCH, M.W., BEVAN, J.A. & SU, C. (1975). Effects of phenoxybenzamine and norepinephrine on transmitter release in the pulmonary artery of the rabbit. *Blood Vessels*, **12**, 122–133.

MCCULLOCH, M.W., RAND, M.J. & STORY, D.F. (1972). Inhibition of ^{3}H-noradrenaline release from sympathetic nerves of guinea-pig atria by a presynaptic α-adrenoceptor mechanism. *Br. J. Pharmac.*, **46**, 523–524P.

NICKERSON, M. & COLLIER, B. (1975). Drugs inhibiting adrenergic nerves and structures innervated by them. In *The Pharmacological Basis of Therapeutics*. ed. Goodman, L.S. & Gilman, A. pp. 533–564. New York: MacMillan Publishing Co.

RAND, M.J., STORY, D.F., ALLEN, G.S., GLOVER, A.B. & MCCULLOCH, M.W. (1973). Pulse to pulse modulation of noradrenaline release through a prejunctional α-receptor auto-inhibitory mechanism. In *Frontiers in Catcholamine Research*. ed. Usdin, E. & Snyder, S.H. p. 579. Oxford: Pergamon Press.

RAND, M.J., MCCULLOCH, M.W. & STORY, F. (1975). Prejunctional modulation of noradrenergic transmission by noradrenaline, dopamine and acetylcholine. In *Central Action of Drugs in Blood Pressure Regulation*. ed. DAVIES, D.S. & REID, J.L. p. 94, Baltimore-London-Tokyo: University Park Press.

STARKE, K. (1972). Influence of extracellular noradrenaline on the stimulation-evoked secretion of noradrenaline from sympathetic nerves; evidence for an α-receptor-mediated feedback inhibition of noradrenaline release. *Naunyn Schmiedebergs Arch. Pharmac.*, **275**, 11–23.

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

STARKE, K., ENDO, T. & TAUBE, H.D. (1975). Relative pre- and postsynaptic potencies of α-adrenoceptor agonists in the rabbit pulmonary artery. *Naunyn Schmiedebergs Arch. Pharmac.*, **291**, 55–78.

STARKE, K., MONTEL, H. & SCHÜMANN, H.J. (1971). Influence of cocaine and phenoxybenzamine on noradrenaline uptake and release. *Naunyn Schmiedebergs Arch. Pharmak.*, **270**, 210–214.

VIZI, E.S., SOMOGYI, G.T., HADHAZY, P. & KNOLL, J. (1973). Effect of duration and frequency of stimulation on the pre-synaptic inhibition by α-adrenoceptor stimulation of the adrenergic transmission. *Naunyn Schmiedebergs Arch. Pharmac.*, **280**, 79–91.

(Received February 26, 1979. Revised March 19, 1979.)