J. Pharm. Pharmac., 1972, **24**, 478

LETTERS TO THE EDITOR

Phenoxybenzamine blockade of neural and exogenous noradrenaline

It is commonly believed that α-adrenoceptor blocking agents antagonize responses to circulating catecholamines more effectively than they antagonize responses to neurally released amines. This opinion originates from experiments with dibenamine and the cat nictitating membrane (Nickerson & Nomaguchi, 1948). Recent investigations have led to controversy over the validity of this viewpoint with respect to vascular smooth muscle (Levin & Beck, 1967; Miranda & Gomez, 1970; Urquilla, Stitzel & Fleming 1970; Bevan & Su, 1971). Of these only Bevan & Su (1971) support the view of Nickerson & Nomaguchi. The following experiments were made in an attempt to resolve some of this controversy.

Helical strips of small mesenteric arteries (1·0 to 1·4 mm o.d.) from mongrel dogs were mounted in standard muscle baths of 10 ml volume, containing modified Krebs-Henseleit solution (mM: NaCl 115·3, KCl 4·6, $CaCl_2$ 1·8, $MgSO_4$ 1·1, $NaHCO_3$ 22·1, glucose 7·8) to which disodium EDTA (0·01 g/litre) had been added. The bathing medium was maintained at 37 ± 0·5° and constantly aerated with 5% carbon dioxide in oxygen. Isometric contractions were monitored via force transducers. A resting tension of 300 mg was applied to the strips and maintained throughout a 2 h equilibration period.

Strips were suspended directly between two electrodes of 18 gauge platinum wire. Square wave pulses of constant voltage (75 V) and duration (0·5 ms) were delivered from a stimulator and the "field" voltage (10 to 14 V in different preparations) was monitored on an oscilloscope. These stimulus parameters release noradrenaline from adrenergic nerves in the arterial wall without directly stimulating smooth muscle cells (Paterson, 1965; Su & Bevan, 1970).

Dose-response curves to noradrenaline and frequency-response curves to electrical stimulation were obtained in each strip. During a frequency-response curve, strips were stimulated in a stepwise fashion without turning off the stimulator and a maximum response was elicited at each frequency before proceeding to the next. An entire frequency-response curve (6 frequencies) required 6 min of stimulation. The noradrenaline stock solution was made by dissolving noradrenaline bitartrate (20 mg/ml) in double-distilled water which contained 0·001 N HCl. Final concentrations of noradrenaline are expressed as g/ml (w/v) of the base. Total volume added to the bath during a noradrenaline dose-response curve was 0·4 ml.

After the tissue had been given several washes over an interval of 30 to 45 min, phenoxybenzamine was added to baths containing test strips and left for ten min while control tissues remained untreated. The phenoxybenzamine stock solution was a parenteral solution of phenoxybenzamine HCl (50 mg/ml) dissolved in distilled water and 25% (v/v) of propylene glycol and ethanol (Smith, Kline & French). Final concentrations of phenoxybenzamine are expressed as g/ml (w/v) of the salt and final concentrations of stock solution solvents (ethanol and propylene glycol) were less than 0·01% (v/v). 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.

Means were compared by Student's *t*-test and considered significant when $P < 0·05$.

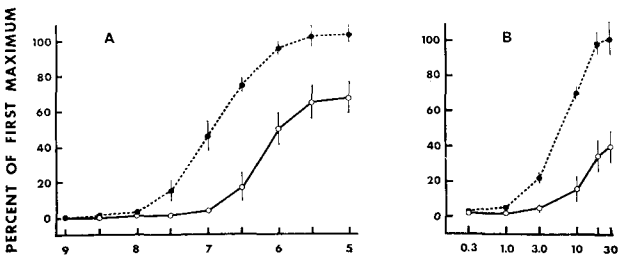

FIG. 1. Effect of phenoxybenzamine (○—○ $1{\cdot}5 \times 10^{-8}$M) on responses to noradrenaline (A) and to transmural stimulation (B). ●--● untreated. Each reponse as expressed as % of the maximum obtained during a control period. The abscissa of (A) is the negative logarithm of the noradrenaline concentration; that of (B) is the frequency of transmural stimulation in Hz. Each point represents the mean of 7 experiments and the vertical bars are the s.e.

In Fig. 1 responses to noradrenaline are expressed as a percent of the maximum tension evoked during a control dose-response curve whereas responses to transmural stimulation are expressed as a percent of the maximum tension evoked during a control frequency-response curve. The dose-response (left panel) and frequency-response (right panel) curves from untreated strips were virtually unaltered from control. The left panel of Fig. 1 illustrates that phenoxybenzamine shifted the noradrenaline dose-response curve to the right and decreased the maximum to $68 \pm 9\%$ of that obtained in the control curve. The effect of phenoxybenzamine on the frequency-response curve to transmural stimulation (right panel) was similar but the maximum response was reduced to $40 \pm 9\%$ of that obtained before phenoxybenzamine treatment. The difference between noradrenaline and transmural stimulation maxima after phenoxybenzamine was statistically significant.

The same data are presented in a different manner in Fig. 2. Responses in all curves in this figure are expressed as a percent of the maximum response evoked by noradrenaline during control dose-response curves. Note in the left panel of Fig. 2 that the dose-response and frequency-response curves obtained from untreated strips are virtually superimposable although transmural stimulation is unable to evoke the same maximum response as exogenously administered noradrenaline. After phenoxybenzamine (right panel) the dose-response curve to noradrenaline is shifted to the right *more* than the frequency-response curve. The difference between the curves in the right panel of Fig. 2 is not statistically significant.

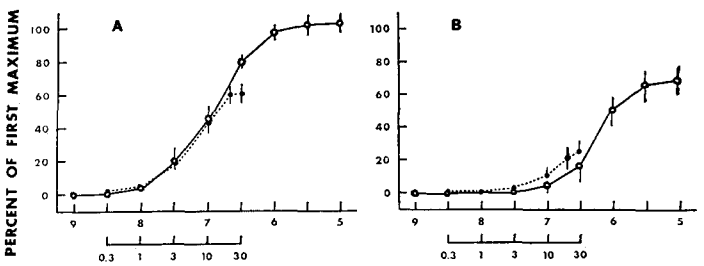

FIG. 2. Effect of phenoxybenzamine on responses to noradrenaline (○—○) and to transmural (●--●) stimulation. Results from untreated tissues are illustrated in panel A and those from phenoxybenzamine treated ($1{\cdot}5 \times 10^{-9}$M) tissues in panel B. Each response is expressed as a % of the maximum response to noradrenaline obtained during a control period. The upper abscissa in each panel is the negative logarithm of the noradrenaline concentration and the lower is the frequency of transmural stimulation in Hz. Each point represents the mean of 7 experiments and the vertical bars are the s.e.

Controversy over the relative ability of α-adrenoceptor blocking agents to inhibit responses to neurally released and exogenous amines may be partially resolved by consideration of the two alternative formats used to illustrate the results of the present experiments. If neurally and exogenously evoked responses are each plotted as a percent of their own maximum response (Fig. 1), it may be concluded that the neurally evoked response is blocked to a greater degree than the response to exogenous noradrenaline. This conclusion supports the view of Urquilla & others (1970), who tested phentolamine in rabbit isolated aorta, and Greenberg (1970) who tested phentolamine and phenoxybenzamine in cat isolated spleen. The format of data presentation used in Fig. 1 is analogous to that used by these authors and could be interpreted that responses to neurally released and exogenous amine are independent of one another, i.e. that two separate populations of α-adrenoceptor are activated by neurally released and exogenous noradrenaline respectively.

The converse is that there is one homogenous population of α-adrenoceptors and exogenous and neurally released amines have equal accessibility to these receptors. Thus, Fig. 2 illustrates the same data with all responses plotted as a percent of the maximum response to exogenous noradrenaline. Fig. 2 suggests the conclusion that responses to either type of stimulus are blocked to an equivalent degree, or, perhaps the response to exogenous amine is slightly more readily blocked. This conclusion supports the view of Bentley & Smith (1967) who reported that neurally and exogenously evoked responses in the rabbit ear artery are blocked equally well by both phentolamine and phenoxybenzamine. Similar results were also reported by McGregor (1965) who studied phenoxybenzamine in perfused mesenteric arteries of rats. These data illustrate that part of the reason for the widely divergent opinion on the ability of α-adrenoceptor blocking agents to block neural and exogenous amine may lie in different initial assumptions on the part of the various investigators.

However, the original view of Nickerson & Nomaguchi (1948), and of Bevan & Su (1971), is not well supported by either of the above methods of presentation. Bevan & Su reported that phenoxybenzamine and phentolamine much more effectively blocked the response to exogenous amine than they blocked the response to neurally released amine in rabbit aorta and pulmonary artery. The format of presentation used in Fig. 2 is analogous to that used by Bevan and Su. In the present experiments (Fig. 2) any difference in blockade is equivocal.

Final resolution of this question awaits clarification of the nature of α-adrenoceptors for neural and exogenous amine. However, this alone may not give a complete answer for phenoxybenzamine clearly has a greater effect on responses to neurally released amine in perfused limb preparations (Levin & Beck, 1967; Miranda & Gomez, 1970) even when the data are analysed in a manner analogous to that used in Fig. 2. In conclusion, it may be stated that the commonly accepted notion that α-adrenoceptor blocking agents more readily block responses to exogenous amines is not justified by the results of the present experiments nor by most other experimental data from vascular smooth muscle preparations.

Supported by USPHS Grant No. HE 13202.

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March 6, 1972

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A common error in brain dopamine assays

It is commonly assumed that L-dopa in acute doses penetrates the blood-brain barrier. However, using the Falck-Hillarp histochemical fluorescence technique, it was shown that after nialamide (250 mg/kg) + L-dopa (75 mg/kg) or L-dopa alone, the amino-acid will not pass the blood-brain barrier due to its extraneuronal decarboxylation to dopamine within the brain capillaries (de la Torre, 1968, 1971). The dopamine remains trapped in the capillary endothelial cell layer where it fluoresces brightly. When nialamide + peripheral dopa decarboxylase inhibitor (PDI) + L-dopa are used, the capillary fluorescence is gradually abolished, correlating with the dose of PDI used. Penetration of L-dopa from capillaries to brain tissue is seen as a bright diffuse fluorescence in the area surrounding the capillary. This progressive penetration of the amino-acid can be mapped out in the brain by inactivating the capillary decarboxylase enzyme with the PDI [Ro 4-4602 N^1 (DL-seryl)-N^2-(2,3,4-trihydroxy benzyl) hydrazine] at doses ranging from 2-50 mg/kg (de la Torre, 1968, 1971).

L-Dopa administered to rats at a dose of 50 mg/kg increases brain dopamine 250% above control values within 30 min. Histochemical fluorescence indicates that in similarly treated rats, the brain capillary fluorescence is markedly intense in both the lumen and endothelial cells (Fig. 1a). In fact, the fluorescence around these capillaries is absent and the normal neuronal fluorescence remains unchanged indicating a lack of penetration by the L-dopa into the brain parenchyma.

If the L-dopa remained in the periphery, it was thought that cerebral perfusion might diminish the total values of brain dopamine.

Intracardiac left ventricular perfusion of cerebral vessels appears to wipe out the fluorescence in the lumen but not that of the capillary endothelial cells (Fig. 1b). Furthermore, perfusion at 15, 30 and 60 min after a standard intraperitoneal injection of L-dopa (50 mg/kg) shows that the dopamine "brain increase" as measured biochemically is reduced 11, 42 and 6% respectively compared to non-perfused treated rats (Fig. 2).

Moreover, if dopamine is injected intraperitoneally in rats, a capillary fluorescence restricted to the lumen is observed. Cerebrovascular perfusion with heparanized saline abolishes the capillary fluorescence almost completely in these animals.

These findings indicate that the blood-brain barrier for dopamine lies in the inner or luminal surface of the capillary which is in contact with the blood. On the other hand, L-dopa appears to penetrate this luminal surface where it appears to enter the endothelial cells or inner capillary layer. This and other data (Bertler, Falck & others 1963, 1966) further suggests that administered L-dopa may be rapidly decarboxylated to dopamine within the endothelial cells of brain capillaries where it is shielded from the saline perfusion.