J. Pharm. Pharmac., 1968, **20**, *Suppl.*, 130S–134S Received May 1, 1968

An adrenergic neuron blocking action of propranolol in isolated tissues

M. D. DAY, D. A. A. OWEN AND P. R. WARREN

Propranolol was tested for adrenergic neuron blocking activity in three isolated sympathetically-innervated smooth muscle preparations; the rat vas deferens, rabbit ileum and rabbit ear artery. In each preparation propranolol impaired the responses to sympathetic stimulation without reducing the responses to added noradrenaline. This blocking action of propranolol resembled that of guanethidine in time of onset and persistence of blocking activity but, unlike blocking by guanethidine, was not reversed by (+)-amphetamine. Desipramine and noradrenaline also failed to reverse the blocking action of propranolol. In the rat vas deferens preparation lignocaine had a weaker and more transient sympathetic blocking action than propranolol. It is suggested that the sympathetic blocking action of propranolol may contribute to its antihypertensive effect in man.

PROPRANOLOL is a potent and specific β-adrenergic receptor blocking agent with little intrinsic sympathomimetic activity (Black, Crowther & others, 1964). Propranolol also has potent local anaesthetic activity (Morales-Aguilerá & Vaughan-Williams, 1965) and clinically has been shown to exhibit antifibrillatory (Rowlands, Howitt & Markman, 1965), anti-anginal (Gillam & Prichard, 1965) and antihypertensive properties (Prichard & Gillam, 1964).

It has been suggested that propranolol lowers arterial blood pressure by impairing cardiac sympathetic tone and thus reducing cardiac output (Prichard, 1968). An antihypertensive agent with this mode of action is of particular interest since it might be free from many side-effects caused by non-selective sympathetic blockade such as occurs with the adrenergic neuron-blocking drugs (Green, 1962). The adrenergic neuron-blocking drugs xylocholine, bretylium and guanethidine have antihypertensive properties in common with propranolol and are potent local anaesthetics (Green, 1962). Propranolol was therefore tested for a possible pre-synaptic blocking action on peripheral adrenergic neurons.

Experimental

Rat isolated vas deferens. Both vasa deferentia removed from recently killed rats were threaded through bipolar platinum electrodes and were set up in organ baths containing aerated Tyrode solution at 32° in separate but simultaneous experiments. Electrical stimulation of the intramural sympathetic nerve endings was with pulses of supramaximal strength (usually 20 V) of 2 msec duration and at a frequency of 5 to 20 pulses/sec delivered from a constant voltage electronic stimulator for periods of 15 sec repeated every 5 min.

Finkleman preparation of rabbit ileum. Preparations were set up and electrically stimulated as described by Day & Rand (1961) except that the Ringer solution was replaced by aerated Tyrode at 37°.

From The Pharmacological Laboratories, Department of Pharmacy, University of Aston, Birmingham 4, England.

Rabbit isolated ear artery preparation. This preparation was set up and electrically stimulated as described by De la Lande & Rand (1965).

Results

Rat isolated vas deferens. In this preparation propranolol (1 to 5 μg/ml) caused a progressive impairment of the responses to sympathetic nerve stimulation whilst the responses to added noradrenaline were either unaffected, or more usually, increased. The result of an experiment in which the sympathetic nerve blocking action of propranolol was compared with that of guanethidine is shown in Fig. 1. In this experiment propranolol (3 μg/ml) caused a similar degree of impairment of the responses to sympathetic stimulation as did guanethidine (1 μg/ml). In each experiment the response to added noradrenaline (2 μg/ml) was slightly increased after establishment of the block. Whereas the adrenergic neuron blocking action of guanethidine was reversed 1 hr after adding (+)-amphetamine (0·05 μg/ml) to the bath (Fig. 1B), this treatment did not restore the responses to sympathetic stimulation after propranolol

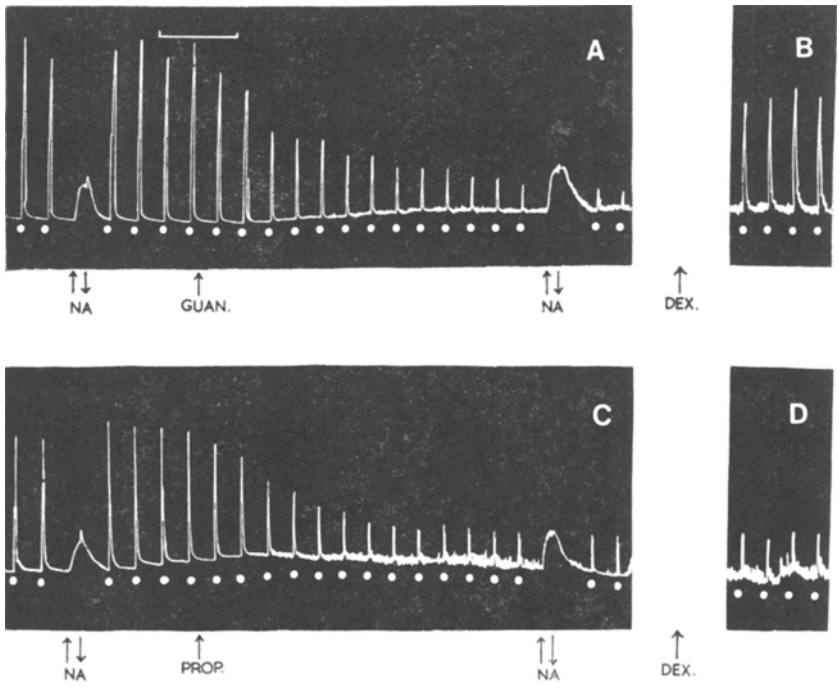

FIG. 1. Rat vas deferens preparations. At white dots stimulation of intramural sympathetic nerves with 2 msec 20 V pulses at frequency of 10 pulses/sec. 2 μg/ml noradrenaline (NA) added to bath at arrows and left in contact with the preparations for 2 min. Upper record: 1 μg/ml guanethidine caused sympathetic block which was partly reversed in B 60 min after adding (+)-amphetamine (DEX) (0·05 μg/ml) to the bath. Lower record: contralateral preparation from same rat sympathetic blockade produced by 3 μg/ml propranolol was not reversed (in D) 60 min after adding (+)-amphetamine to the bath.

(Fig. 1D). The adrenergic neuron blocking action of propranolol was persistent and was only very slowly reversed by repeated washing of the preparation over several hours.

In other experiments, attempts were made to reverse the blocking action of propranolol with either noradrenaline (1 to 2 μg/ml) or desipramine (0·1 to 0·5 μg/ml). These concentrations of noradrenaline initially contracted the tissue but caused no increase in the sympathetic responses after propranolol left in contact for up to 45 min. Desipramine caused a large increase in the sensitivity to added noradrenaline but had no effect on the response to sympathetic stimulation when added before or after the establishment of a propranolol block.

In a few preparations pronethalol was used instead of propranolol and was found to have a similar action in blocking nervously-mediated responses without reducing the responses to added noradrenaline. Pronethalol was approximately half as potent as propranolol in producing nerve block and was more readily reversed by washing.

Finkleman preparation. This preparation was chosen to test the effects of propranolol on inhibitory sympathetic responses because the responses are mediated by an action of neuronal noradrenaline on both α- and β-adrenergic receptors (Furchgott, 1960). The results using this preparation were essentially the same as those obtained using the isolated vas deferens preparation. Thus, propranolol (3 μg/ml) produced a similar impairment of the responses to sympathetic nerve stimulation as did guanethidine (1 μg/ml). Fig. 2 illustrates an experiment in which propranolol (3 μg/ml) produced a rapidly developing impairment of the responses to sympathetic stimulation although the inhibitory responses to added noradrenaline were virtually unaffected. As in the vas deferens preparation, the blocking action of propranolol was not reversed by (+)-amphetamine (0·1 to 0·5 μg/ml) and was only slowly reversed by repeated washing of the preparation. The blocking action of guanethidine was even more persistent after washing the preparation but was readily

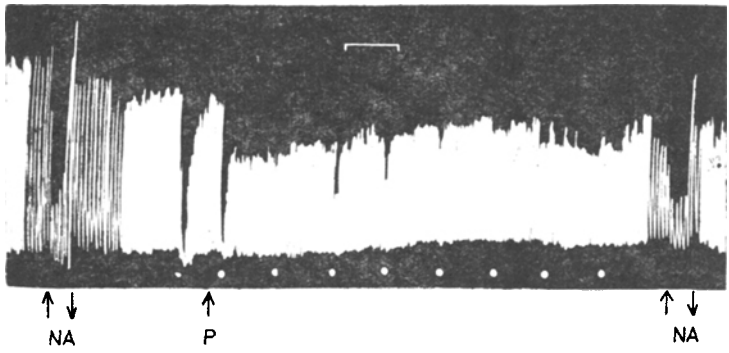

FIG. 2. Finkleman preparation of rabbit ileum. At white dots periarterial sympathetic nerves stimulated with 2 msec 10 V pulses at frequency of 50 pulses/sec. Noradrenaline 0·05 μg/ml added to bath (at NA) and left in contact with preparation 30 sec. Propranolol 3 μg/ml (at P) added to bath. Drum speed increased during noradrenaline responses.

reversed by (+)-amphetamine. Pronethalol had a similar effect in this preparation to propranolol but again was less potent, was more easily reversed, and itself inhibited the spontaneous activity of the preparation.

Rabbit isolated ear artery preparation. This preparation was chosen to determine whether propranolol had a similar adrenergic neuron blocking action on sympathetically innervated vascular smooth muscle as it did in other smooth muscle preparations tested, since this may have some bearing on its use as an antihypertensive agent. It was found that propranolol (0·25 to 1 μg/ml) produced a slowly-developing but persistent impairment of the constrictor responses to sympathetic stimulation whereas the responses to injected noradrenaline were enhanced. In this preparation, unlike the other preparations tested propranolol was at least as potent as guanethidine in producing adrenergic neuron blockade.

Comparison of the nerve blocking actions of propranolol and lignocaine. Propranolol has similar local anaesthetic potency to lignocaine (Morales-Aguilerá & Vaughan-Williams, 1965) and it was thought possible that this action could explain its effects on adrenergic neurons. For this reason the blocking action of propranolol was compared with that of lignocaine in the Finkleman preparation of rabbit ileum and in the rat isolated vas deferens. In the rabbit ileum preparation lignocaine usually caused impairment of the pendular movements of the preparation in concentrations (10 to 30 μg/ml) which did not significantly affect the responses to sympathetic stimulation. Propranolol on the other hand caused a complete abolition of the nervously mediated responses at a concentration of 1 to 3 μg/ml which did not affect the spontaneous activity of the preparation.

In the isolated vas deferens preparation lignocaine did not affect the responses to sympathetic stimulation at a concentration (30 μg/ml) ten times higher than that of propranolol needed to cause an almost complete block of the responses. At a concentration of 50 to 100 μg/ml, lignocaine caused a partial nerve blockade which unlike the propranolol block was readily reversed by washing.

Discussion

The results described indicate that propranolol has a potent blocking action on adrenergic sympathetic neurons in isolated smooth muscle preparations. The adrenergic neuron blocking action of propranolol appears to be pre-synaptic and independent of its post-synaptic effect on β-adrenergic receptors. Thus, at a time when the block was at a maximum the responses to exogenous noradrenaline were either unaffected or increased; in addition the block occurred in tissues such as the rat vas deferens and rabbit ear artery in which only α-adrenergic receptors are involved.

The potency of propranolol in blocking adrenergic neurons was only slightly less than that of guanethidine to which it has a similar time of onset and was almost equally persistent in its blocking action after changing the bath fluid. However, the blocking action of propranolol could be distinguished from that of guanethidine by the fact that only that of

guanethidine was reversed by (+)-amphetamine. Antagonism occurs with (+)-amphetamine and other adrenergic neuron blocking agents and is probably competitive in nature (Day, 1962; Day & Rand, 1963). Similarly it is unlikely that the blocking action of propranolol is caused by depletion of noradrenaline from the sympathetic nerves, as occurs with reserpine, since the block was not reversed by noradrenaline. Desipramine was tested as a potential propranolol antagonist because of the recent report that it partially antagonized the action of propranolol in preventing the increase in rate of beating of isolated atria in response to sympathetic stimulation (Shimamoto & Toda, 1968). No such antagonism was found in the rat vas deferens preparation despite a large increase in sensitivity of the preparation to added noradrenaline caused by desipramine.

Thus the most likely explanation of the blocking action of propranolol is to be found in its potent local anaesthetic property. However, in a direct comparison with lignocaine, with which it has been reported to be approximately equipotent as a local anaesthetic (Morales-Aguilerá & Vaughan-Williams, 1965), propranolol was found to be much more potent and persistent in its blocking action on adrenergic neurons. We cannot preclude the possibility that the sympathetic blocking action of propranolol is a consequence of its local anaesthetic activity since it may be that it exerts this action on sympathetic nerve endings more effectively than lignocaine possibly as a result of more complete penetration into the tissue.

The antihypertensive effect of propranolol in man is of slow onset (Prichard & Gillam, 1964) and this is consistent with the hypothesis that the drug is slowly accumulated in peripheral adrenergic neurons thus causing a reduction in sympathetic vasomotor tone which would tend to reinforce its better known β-blocking action on cardiac receptors in lowering arterial blood pressure.

Acknowledgements. We wish to thank I.C.I. Ltd. for generous gifts of propranolol and pronethalol, the Medical Research Council for a grant, and Mr. A. Richardson for technical assistance.

References

Black, J. W., Crowther, A. F., Shanks, R. G., Smith, L. H. & Dornhorst, A. C. (1964). *Lancet*, **1**, 1080–1081.
Day, M. D. (1962). *Br. J. Pharmac. Chemother.*, **18**, 421–439.
Day, M. D. & Rand, M. J. (1961). *Ibid.*, **17**, 245–260.
Day, M. D. & Rand, M. J. (1963). *Ibid.*, **20**, 17–28.
De La Lande, I. S. & Rand, M. J. (1965). *Aust. J. exp. Biol. med. Sci.*, **43**, 639–659.
Furchgott, R. F. (1960). *Adrenergic Mechanisms*, pp. 246–252, Ciba Symposium. Editors: Vane, J. R., Wolstenholme, G. E. W. & O'Connor, M. London: Churchill.
Gillam, P. M. S. & Prichard, B. N. C. (1965). *Br. med. J.*, **2**, 337–339.
Green, A. F. (1962). *Adv. Pharmac.*, **1**, 161–225.
Morales-Aguilerá, A. & Vaughan-Williams, E. M. (1965). *Br. J. Pharmac. Chemother.*, **24**, 332–338.
Prichard, N. C. (1968). *Practitioner*, **200**, 30–38.
Prichard, N. C. & Gillam, P. M. S. (1964). *Br. med. J.*, **2**, 725–727.
Rowlands, D. J., Howitt, G. & Markman, P. (1965). *Ibid.*, **1**, 891–892.
Shimamoto, K. & Toda, N. (1968). *Br. J. Pharmac. Chemother.*, **32**, 539–545.