[^{3}H]-clonidine bound membranes prepared from the renal cortex bound 76.55 ± 1.25%, those from the medulla 18.70 ± 0.36% and those from the papilla 4.75 ± 1.37% (n = 4). More detailed analysis of binding, performed on membranes prepared from the renal cortex and medulla, showed that the association of [^{3}H]-clonidine to the binding site was rapid with a $T_{\frac{1}{2}} \ll 2$ min at 25°C and that binding had equilibrated within 20 min of the start of incubation. The binding isotherm showed that the process was saturable. Scatchard analysis of [^{3}H]-clonidine binding to membranes from renal cortex revealed that binding was to a single class of sites ($P < 0.001$ in all cases) with a dissociation constant (K_d) of 9.03 ± 0.76 (n = 4) and that the density of binding sites was 21.6 ± 1.7 p.mol/g wet wt. tissue. Hill plots of the data obtained in these experiments were linear ($P < 0.001$ in all cases) with a mean Hill coefficient of 1.02 ± 0.02 (n = 4) indicating the absence of cooperative site interactions in the binding of [^{3}H]-clonidine to its receptor site. Membranes prepared from renal medulla bound less [^{3}H]-clonidine than those from renal cortex. The K_d for [^{3}H]-clonidine binding in membranes from the medulla was 4.4 ± 0.9 nM and the density of binding sites 3.9 ± 1.0 p.moles/g wet wt. (n = 3).

The results indicate that in the guinea pig kidney [^{3}H]-clonidine binds to an α-adrenoceptor which is located primarily in the renal cortex.

Supported by a grant from the National Health and Medical Research Council of Australia.

References

SUMMERS, R.J., JARROTT, B. & LOUIS, W.J. (1978a). The characteristics of [^{3}H]-clonidine binding to membranes from guinea pig kidney. *Proc. Aust. Phys. Pharmacol. Soc.*, **9**, 90P.

SUMMERS, R.J., JARROTT, B. & LOUIS, W.J. (1978b). [^{3}H]-clonidine binding to α-adrenoceptors in guinea pig kidney membranes in "Recent advances in the pharmacology of adrenoceptors", Elsevier, North Holland, in press.

U'PRICHARD, D.C., GREENBERG, D.A. & SNYDER, S.H. (1977). Binding characteristics of a radiolabelled agonist and antagonist at central nervous system alpha noradrenergic receptors. *Mol. Pharmacol.*, **13**, 454–473.

Time course of the pre- and post-junctional effects of clonidine in the pithed rat

J.R. DOCHERTY & J.C. McGRATH

Institute of Physiology, University of Glasgow, Glasgow G12 8QQ, Scotland

In the pithed rat clonidine inhibits the chronotropic response to stimulation of the cardiac sympathetic nerves by a pre-junctional α-adrenoceptor agonism and raises blood pressure by a post-junctional α-adrenoceptor agonism (Armstrong & Boura, 1975; Drew, 1976). It has been suggested that the latter is faster in onset and shorter in duration than the former (Cavero, Gomeni, Lefevre & Roach, 1977). We have compared the time courses of the pre- and post-junctional effects of clonidine in several tissues and correlated these with plasma levels of clonidine.

Male rats were pithed and respired with 100% O_2. Gallamine (20 mg/kg) was given except where cardioaccelerator responses were examined (Docherty & McGrath, 1977a). Arterial blood pressure, heart rate and, where appropriate, longitudinal isometric tension of anococcygeus and vas deferens (Gillespie & McGrath, 1973, 1974) were monitored. The optimal sympathetic outflow for each tissue was stimulated via the pithing rod (0.05 – 1 ms, supramaximal pulses) (Gillespie & McGrath, 1973, 1974; Docherty & McGrath, 1977a). Blood samples were taken at 1, 5, 15 and 40 min after clonidine injection and plasma was assayed for clonidine (Draffin, Clare, Murray, Bellward, Davies & Dollery, 1976).

The rates of onset of the effects of clonidine were tested while continuously stimulating the cardiac sympathetic nerves at 0.1 Hz. Under these conditions the heart rate is elevated to a sub-maximal plateau but no endogenous α-adrenoceptor feedback is present (Docherty & McGrath, 1977b). The onset of both cardiac inhibitory and pressor effects were as rapid as could be expected from equilibration of clonidine with the tissues. In fact the fall in heart rate caused by clonidine was as quick as could be accomplished by ceasing electrical stimulation.

The duration of clonidine's effects were studied while applying intermittent sympathetic stimulation. The inhibitory effects against responses mediated by adrenergic nerves in heart, anococcygeus and vas deferens had similar time courses e.g. the tachycardia to a single pulse was reduced to 1.7% of control in 0.5 min by clonidine (5 μg/kg) and had recovered to 50% of control by 25 minutes. The decline in plasma levels of clonidine correlated well with recovery of

the sympathetic responses. In contrast the pressor response reached its peak within 0.5 min but had disappeared by 8 min although the plasma levels of clonidine were virtually unchanged over this period and the response to a subsequent injection of clonidine or noradrenaline was unaffected. Similarly in the anococcygeus the contraction by clonidine was shorter-lived than the presynaptic inhibitory effect.

In conclusion, (i) all monitored effects of clonidine had an equally rapid onset, (ii) the duration of prejunctional inhibition was related to plasma clonidine levels, (iii) the short-lived post-junctional effects were related to the initial high concentration due to the injection of a bolus.

Supported by the Medical Research Council. We would like to thank Elizabeth Neill and Stephen Jones of the Royal Postgraduate Medical School, Hammersmith Hospital for analysis of plasma clonidine.

References

ARMSTRONG, J.M. & BOURA, A.L.A. (1973). Effects of clonidine and guanethidine on peripheral sympathetic nerve function in the pithed rat. *Br. J. Pharmac.*, **47**, 850–852.

CAVERO, I., GOMENI, R., LEFEVRE, F. & ROACH, A.G. (1978). Time course analysis of the cardiovascular effects of clonidine resulting from the activation of cardiac pre- and vascular postsynaptic α-adrenoceptors in the pithed rat. *Br. J. Pharmac.*, **62**, 468P.

DOCHERTY, J.R. & McGRATH, J.C. (1977a). Potentiation of cardiac sympathetic responses *in vivo* by pancuronium bromide. *Br. J. Pharmac.*, **61**, 472–473P.

DOCHERTY, J.R. & McGRATH, J.C. (1977b). Effect of presynaptic α-adrenoceptor blockade on the chronotropic response to cardiac sympathetic nerve stimulation in the pithed rat. *J. Physiol. Lond.*, **273**, 63–64P.

DRAFFAN, G.H., CLARE, R.A., MURRAY, S., BELLWARD, G.D., DAVIES, D.S. & DOLLERY, C.T. (1976). In *Advances in Mass Spectrometry in Biochemistry and Medicine.* Vol. 2, ed. Frigerio, A. pp. 389–394. New York: Spectrum Publications.

DREW, G.M. (1976). Effects of α-adrenoceptor agonists and antagonists on pre- and postsynaptically located α-adrenoceptors. *Eur. J. Pharmac.* **36**, 313–320.

GILLESPIE, J.S. & McGRATH, J.C. (1973). The spinal origin of the motor an inhibitory innervation of the rat anococcygeus muscles. *J. Physiol. Lond.*, **230**, 659–672.

GILLESPIE, J.S. & McGRATH, J.C. (1974). The effect of pithing and of nerve stimulation on the depletion of noradrenaline by reserpine in the rat anococcygeus muscle and vas deferens. *Br. J. Pharmac.*, **52**, 585–590.

Estimation of dissociation constants and relative efficacies of isoprenaline, orciprenaline and terbutaline in guinea-pig isolated atria by use of functional antagonism

K.J. BROADLEY & C.D. NICHOLSON

Department of Applied Pharmacology, Welsh School of Pharmacy, University of Wales Institute of Science and Technology, King Edward VII Avenue, Cardiff CF1 3NU

Affinity values for antagonists indicate that the β-adrenoceptors mediating positive inotropic and chronotropic responses of isolated atria are identical (Blinks, 1967; Lumley & Broadley, 1975). However, agonists exhibit selective chronotropic activity and partial agonists have lower maxima for the tension response (Lumley & Broadley, 1977). To determine the possible source of these differences, both dissociation constants and relative efficacies must be determined (Jenkinson, 1973). We have used functional antagonism (Van den Brink, 1973; Buckner, Torphy & Costa, 1978) in isolated guinea-pig atria to calculate these parameters for isoprenaline, orciprenaline and terbutaline.

Rate responses were obtained from isolated right atria and tension responses from paced left atria (2 Hz), set up at 38°C in Krebs-bicarbonate solution gassed with 5% CO_2 in O_2. Cumulative dose-response curves to isoprenaline were followed by orciprenaline or terbutaline curves. The mean (n = 4) orciprenaline (100.5 ± 3.99%) and isoprenaline rate maxima did not differ significantly, but the orciprenaline tension (92.3 ± 0.35%) and terbutaline rate (84.4 ± 4.8%) and tension (58.2 ± 2.62%) maxima were significantly less ($P < 0.05$). These terbutaline maxima differed significantly ($P < 0.01$).

To determine the dissociation constants of these agonists, cumulative dose-response curves to isoprenaline and either orciprenaline or terbutaline were constructed before and in the presence of carbachol (200 nM) as the functional antagonist. Responses were measured as increase above their own resting levels and plotted as a percentage of the pre-carbachol isoprenaline maximum against log molar concentration. All experiments were corrected for sensitivity changes from control experiments (n = 4). The affinities were obtained from double reciprocal plots of equiactive molar concentrations before and after carbachol. These yielded the dissociation constant K_a as slope –