

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

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Clonidine administered intravenously to pithed rats produces hypertension and a reduction in the cardiac acceleration evoked by electrical stimulation of the thoracic sympathetic outflow (Armstrong & Boura, 1973). Whilst the vascular action of clonidine is attributed to an activation of α -adrenoceptors present in the arterial vascular wall, the effect on the cardiac pacemaker is believed to be due to clonidine stimulating α -adrenoceptors located on noradrenergic neuronal endings which results in a reduced release of noradrenaline (Langer, 1977).

In pithed male Charles River rats given i.v. atropine sulphate (1.0/mg kg) and (+)-tubocurarine (5.0 mg/kg) a sustained tachycardia of about 90–110 bts/min was evoked by continuously stimulating the thoracic spinal cord (0.3–0.7 Hz, 0.5 ms, 60–70 V). The changes in carotid diastolic blood pressure (BP) and heart rate (HR) produced by 1.0 and 5.0 μ g/kg clonidine as well as the time (t) at which they occurred were read from the traces and the rate constants of onset and decay of these two effects were then determined using a biexponential equation (Gibaldi & Perrier, 1975): $\Delta BP (\Delta HR) = -A \exp(-\alpha t) + B \exp(-\beta t)$. The ratios $0.693\alpha^{-1}$ and $0.693\beta^{-1}$ respectively give the half lives of the appearance and disappearance of BP and HR responses. The times (t_{max}) at which the BP (ΔBP_{max}) and HR (ΔHR_{max}) reached their peaks were obtained from the original data.

The continuous electrical stimulation of the thoracic spinal cord produced a tachycardia which remained constant for at least 60 minutes. The hypertension and the fall in the heart rate produced by clonidine were well described by the exponential function utilized for the analysis of the data. The peak changes in heart

rate (-28.4 ± 1.9 and -57.2 ± 5.2 bts/min after 1.0 and 5.0 μ g/kg clonidine, respectively) occurred within 120 and 150 s after the clonidine injections, whereas the hypertension reached its maximum in approximately 10 seconds. Therefore, the rate constant of the onset (α) of the vascular effect of clonidine was 8–9 times greater than that of the fall in heart rate. Similarly, the cardiac effects of clonidine recovered to control levels much later than the vascular effects. The times required for a 50% recuperation of the peak changes in heart rate ($t_{1/2}$) were 406 and 835 s after 1.0 and 5.0 μ g/kg clonidine respectively, whilst the $t_{1/2}$ values for the clonidine pressor responses were 58 and 88 seconds.

These results indicate that in the pithed rat with an experimentally induced sympathetic tachycardia clonidine in small doses was able to mediate sustained decreases in heart rate via activating presynaptic α -adrenoceptors. The kinetic profile of this clonidine effect is entirely different from that characterizing the hypertensive response implying that the general classical analysis using only the peak changes in diastolic carotid blood pressure and heart rate (Drew, 1976) is unsuitable to evaluate the relative potencies of clonidine and other similar compounds as agonists of cardiac pre- and vascular postsynaptic α -adrenoceptors.

Finally, the complete time-course analysis of the cardiovascular effects of clonidine may underline a structural difference between the cardiac pre- and the vascular postsynaptic α -adrenoceptors; it would also provide a better methodology to evaluate the relative potencies of compounds possessing pre- and postsynaptic α -adrenoceptors stimulant properties.

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The effects of prazosin on the clonidine induced hypotension and bradycardia in rats and sedation in chicks

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Clonidine produces centrally mediated hypotension,

bradycardia and sedation which are inhibited by various α -adrenoceptor antagonists (Delbarre & Schmitt, 1971; Schmitt, Schmitt & Fénard, 1973). Whilst these cardiovascular effects of clonidine are attributed to an activation of central postsynaptic α -adrenoceptors (Haeusler, 1974), the clonidine sedation has been postulated to result from an action on presynaptic α -adrenoceptors or at least receptors resembling peripheral presynaptic α -adrenoceptors

(Żebrowska-Lupina, Przegaliński, Sloniec & Kleinrok, 1977; Drew, Gower & Marriott, 1977).

We have investigated the modification of the centrally mediated cardiovascular effects of clonidine by using prazosin, a relatively selective antagonist of postsynaptic α -adrenoceptors in the peripheral cardiovascular system of the rat (Cavero, Lefèvre & Roach, 1977).

In 8 urethane (1.5 g/kg, i.p.) anaesthetised normotensive Charles River rats clonidine (10.0 μ g) injected into the lateral cerebral ventricles (c.v.) induced peak falls in mean arterial blood pressure (MABP) and heart rate (HR) of 23.5 ± 2.8 mmHg and 66.9 ± 10.1 bts/min which occurred respectively 20 and 15 min after its injection (initial MABP and HR levels: 94.5 ± 7.0 mmHg, 363.0 ± 11.0 bts/min). In another group of normotensive rats i.c.v. or i.v. prazosin (25 μ g) lowered blood pressure to a level which was not significantly different from that achieved after i.c.v. clonidine. This problem was avoided by administering prazosin orally for 3 days (first day, 1.0 and 0.5 mg/kg; second and third days 2×0.5 mg/kg). The blood pressures (93.3 ± 4.5 mmHg, $n = 9$) and heart rates (354.7 ± 15.3 bts/min, $n = 9$) of these animals after inducing urethane anaesthesia 12 h after the last dose of prazosin were not significantly different from those of the untreated rats. In these prazosin pretreated animals, the hypotensive and bradycardic effects of clonidine (10.0 μ g, i.c.v.) were almost entirely abolished.

In two day old chicks, clonidine (0.5, 1.0 and 2.0 mg/kg, i.m.) induced a sleep-like state, the duration of which was a function of the dose (14.8 ± 1.6 , 20.8 ± 1.2 and 32.0 ± 2.9 min, respectively; $n = 14$ /group). These effects were reduced (3.2 ± 1.3 , 7.7 ± 0.6 and 11.9 ± 0.9 min; $n = 10$) when clonidine was given 10 min after 15.0 mg/kg, i.p. phenolamine, an antagonist of pre- and postsynaptic α -adrenoceptors. In contrast, the same dose of prazosin

tended to increase the clonidine hypnosis in chicks (16.1 ± 1.2 , 25.2 ± 3.0 and 41.7 ± 3.0 min; $n = 12$).

In conclusion, oral prazosin antagonized the clonidine induced hypotension and bradycardia in rats probably by inhibiting receptors which possess similar characteristics to vascular postsynaptic α -adrenoceptors. In contrast, the lack of activity of prazosin in preventing clonidine hypnosis in chicks may indicate that the receptors responsible for evoking this effect may respond in a similar way as the rat cardiac presynaptic α -adrenoceptors to agonists or antagonists.

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A simple and cheap vacuum filtration

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Filtration under reduced pressure is a technique which has been applied in studies where speed of separation and rapid washing of trapped material are desirable. Described here is a simple, cheap and effective manifold for separating subcellular particles from in-

cubation medium which has been used in this laboratory for uptake, release and receptor binding studies.

Six filtration units as shown in Figure 1 are connected by 5 cm lengths of 22 mm copper tube. The Swinnex 25 mm filter support is pressed onto the side arm of the 'T' piece using a vice after the Luer needle attachment and stem have been drilled out with a 12 mm bit. Twenty-two mm Yorkshire 'elbows' at the corners and 22 mm copper tubing is used to complete a ring structure. Another 22 mm Yorkshire 'T' piece is placed at a convenience point in the 'ring', and this latter 'T' piece has the side arm fitted with a 22 mm to 7 mm reducer, into which is soldered a short length of 7 mm copper tubing. All other connections are made with rubber pressure tubing. The manifold is connected by a three way glass tap to a 4 litre vacuum