Short communications

Effects of clonidine and guanethidine on peripheral sympathetic nerve function in the pithed rat

J. M. ARMSTRONG AND A. L. A. BOURA

Pharmacology Laboratory, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent, BR3 3BS

Clonidine, in low intravenous doses, inhibited the increased heart rate of pithed rats caused by peripheral sympathetic nerve stimulation. The magnitude of this effect was greatest at low frequencies of nerve stimulation, responses to high frequencies being little affected by the drug. In contrast, guanethidine reduced cardiac responses to both low and high rates of nerve stimulation. The difference between the depressant effects of the two drugs on responses to various frequencies of sympathetic nerve traffic may contribute to the differences known to occur between their properties as hypotensive agents.

Dhasmana, Fokker & Spilker (1972) have recently reported that the centrally acting hypotensive drug clonidine did not inhibit peripheral sympathetic nerve function in the pithed rat at dose levels which lowered arterial blood pressure in anaesthetized animals. They found that, in contrast to the effects of the peripheral blocking agent guanethidine, a relatively large dose of clonidine was required to reduce pressor responses to a physiologically high frequency (10 Hz) of sympathetic nerve stimulation using the technique of Gillespie, Maclaren & Pollock (1970) to stimulate the spinal autonomic outflow. They suggested that their findings supported the contention that the site of the hypotensive action of the drug was within the central nervous system and proposed that this technique could be used to help differentiate between centrally and peripherally acting hypotensive agents. We therefore think it of interest to describe results which show that clonidine does depress peripheral sympathetic nerve function in the pithed rat, after administration of hypotensive doses, providing that low frequencies of nerve stimulation are used.

Methods.—Male Wistar rats (250–300 g) were pithed, during a brief period of ether anaesthesia. The pithing rod was used to stimulate the spinal sympathetic outflow, in the manner described by Gillespie *et al.* (1970), except that both electrodes were of silver ribbon and mounted 2·5 mm apart approximately 1 cm from the end of the rod ; this minimized spread of current to voluntary nerves. Movement of skeletal muscles was further reduced by intravenous tubocurarine chloride (1 mg/kg). The animals were artificially respired (50 strokes/min, 1 ml/100 g) and the rectal temperatures maintained at 38° C. When the electrodes were close to the origin of the sympathetic cardiac nerves (C7–T1) stimulation caused tachycardia with relatively little change in blood pressure. Arterial pressure was measured by means of a transducer connected to the right common carotid artery and integrated heart rate was recorded using the pressure wave to trigger a ratemeter. Both cardiovascular variables were displayed on a Devices M2 physiological recorder. The stimulation was supramaximal (approximately 70 V) and the pulse width 0·5 mseconds. A logarithmic series of progressively increasing stimulus frequencies was applied, each frequency being continued until the maximum increase in heart rate had been obtained (usually within 2 minutes).

Drugs were injected into the femoral vein in a logarithmic series of increasing doses, the series of nerve stimulations being applied before and 10 min after each dose. Saline (0·9% w/v NaCl solution) was injected intravenously into further animals for control purposes.

Results.—Stimulation of the sympathetic cardiac outflow in this preparation gave an extremely sensitive measure of peripheral sympathetic nerve function. The effect of a single shock could readily be detected, the heart rate usually increasing by 25 beats/min above the basal rate of approximately 350 beats/minute. A stimulus frequency of 0·05–0·25 Hz caused a phasic increase in heart rate, fusion of the responses occurring at 0·25–0·5 Hz.

Clonidine given intravenously at dose levels greater than 10 μg/kg reduced the tachycardia caused by sympathetic nerve stimulation, the magnitude of the inhibition being inversely proportional to the stimulus frequency (Fig. 1a). After 10 μg/kg clonidine there was some reduction in the mean responses, especially to the
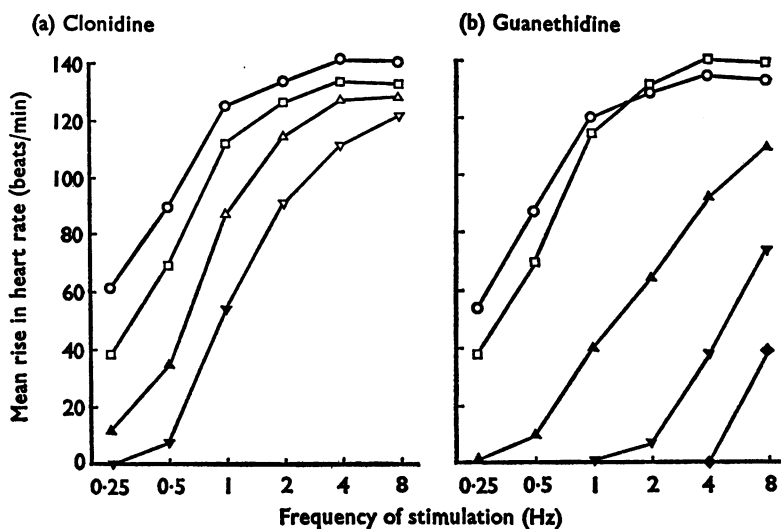
FIG. 1. Maximum tachycardia caused by preganglionic cardiac nerve stimulation at various frequencies. Each point represents the mean of observations taken from 5–6 rats. (a) Effect of clonidine hydrochloride i.v.: ○——○, initial; □——□, 10 μg/kg; △——△, 30 μg/kg; ▽——▽, 100 μg/kg. (b) Effect of guanethidine sulphate i.v.; ○——○, initial; □——□, 0·25 mg/kg; △——△, 0·5 mg/kg; ▽——▽, 1·0 mg/kg; ◇——◇, 2·0 mg/kg. A filled symbol indicates a mean response differing significantly (P<0·05) from the initial.

lower frequencies, but at no frequency was the magnitude of the response significantly different from that obtained initially (P> 0·05). When the dose was increased to that which Dhasmana et al. (1972) found to be hypotensive (30 μg/kg) the cardiac responses were inhibited still further, the depression of the responses to 0·25 Hz and 0·5 Hz being significant (P=0·02). A dose of 100 μg/kg significantly reduced the responses to the three lowest frequencies of nerve stimulation (P=0·01−0·001), blocking completely the increased heart rate caused by 0·25 Hz.

In contrast guanethidine depressed responses to all frequencies of nerve stimulation (Fig. 1b) and caused an approximately parallel movement to the right of the frequency–response curves. Some reduction in the mean responses to nerve stimulation occurred after intravenous doses of 0·25 mg/kg but this was not significant (P>0·05). However, after administration of the higher doses (0·5− 2·0 mg/kg), responses to all frequencies of nerve stimulation were significantly depressed (P=0·05−0·001).

When inhibition of the cardiac response, to indirect stimulation at 0·25 Hz, was plotted against the log of the dose administered, the mean slope (±s.d.) of the regression line obtained with clonidine (59±11·9) was found to be significantly less

(P=0·001) than that obtained using guanethidine (123±15·6).

Discussion.—These findings show that in the rat clonidine is capable of depressing cardiac responses to peripheral sympathetic nerve stimulation at doses previously reported to just lower arterial blood pressure. This effect may result from specific inhibition of noradrenaline release at the adrenergic nerve endings as observed by Starke, Wagner & Schümann (1972) in studies using the rabbit isolated heart. Our finding that the slope of the regression line, relating inhibition of sympathetic cardiac nerve function to the dose of drug administered, was much less for clonidine than for guanethidine is in keeping with the suggestion of these workers that the two drugs block adrenergic transmission by dissimilar mechanisms.

Our conclusions contrast with those of Dhasmana et al. (1972) but this apparent inconsistency is capable of explanation. In our experiments only cardiac responses to frequencies of sympathetic nerve stimulation of 0·25−1·0 Hz were depressed by clonidine, those resulting from the use of higher frequencies (2–8 Hz) being not significantly changed. Dhasmana et al. (1972) stimulated nerves at 10 Hz and in keeping with our results found no inhibition by clonidine. Moreover they also

studied the effects of drugs on branches of the sympathetic outflow (T7—T9) other than the cardiac nerves and there is some evidence that the cardiac adrenergic nerves may be more susceptible to depression by blocking agents than some other peripheral sympathetic nerves (Armstrong & Boura, 1970).

The hypotension occurring after the administration of clonidine is generally thought to be due to a central action which reduces the rate of bulbar sympathetic efferent discharge although a peripheral adrenergic neurone blocking action may contribute (Werner, Starke & Schümann, 1972). The exact mechanism responsible for the lowering of central sympathetic tone remains in doubt. It has been postulated that it is due to stimulation of central postsynaptic α-adrenoceptors (Schmitt, 1970) but recent evidence supports the suggestion that a pre-synaptic noradrenergic neurone blocking action may be responsible (Briant & Reid, 1972). It therefore appears that clonidine's ability to lower sympathetic efferent tone may be the result of an action on central noradrenergic neurones which is similar and additional to that causing depression of peripheral adrenergic nerve transmission. Preferential depression of low frequencies of adrenergic transmission within the central nervous system would explain how clonidine reduces resting and low frequencies of evoked sympathetic efferent nerve traffic whilst allowing passage of high rates under conditions of cardiovascular stress (Schmitt, 1970). Blockade of only the lower rates of adrenergic nerve transmission, both centrally and peripherally, would also explain why postural and exertional hypotension is minimal with this drug. An analogous suggestion has previously been put forward in more detail to account for differences in the pro-

minence of these effects occurring between the peripheral sympathetic blocking agents bretylium and guanethidine (Boura & Green, 1962).

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