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Effects of the vasodilator endralazine given with the antihypertensive agent guanfacine on heart rate and blood pressure of spontaneously hypertensive rats and normotensive dogs

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Endralazine (BQ 22-708, Miretilan) and guanfacine (BS 100-141, Estulic) were tested alone and in combination for their effects on blood pressure and heart rate in conscious spontaneously hypertensive rats and conscious normotensive dogs. During combined administration of endralazine and guanfacine to spontaneously hypertensive rats, guanfacine 1 mg kg^{-1} i.v. blocked the tachycardia caused by endralazine, 1 mg kg^{-1} i.v. After oral administration, the tachycardia induced by 0·5 and 1 mg kg^{-1} of endralazine was inhibited dose-dependently by 0·05, 0·1 and 0·5 mg kg^{-1} of guanfacine. The antihypertensive efficacy of endralazine was either unchanged or increased by guanfacine at 0·05–1 mg kg^{-1}. In normotensive conscious dogs, 0·2 mg kg^{-1} i.v. of guanfacine antagonised the tachycardia elicited by 0·3 mg kg^{-1} i.v. of endralazine. The elimination of the endralazine-induced reflex tachycardia by guanfacine suggests that the combination of both drugs could be useful in antihypertensive therapy.

The pyridopyridazine derivative endralazine (BQ 22-708, Miretilan) has been characterized experimentally as an antihypertensive agent with a peripheral vasodilator activity and a reflex tachycardia (Salzmann et al 1979; Schenker & Salzmann 1979). Clinical findings confirmed the antihypertensive effect of endralazine in man (Lehmann et al 1976, 1977a,b, 1978a,b; Kirch & Distler 1977; Reubi 1978; Hitzenberger & Stumpe 1980; Kindler et al 1981). In these studies, a reduction of peripheral resistance is assumed to be an important cause of the fall in blood pressure (Lehmann et al 1976, 1977a,b).

Guanfacine (BS 100-141, Estulic) is a guanidine derivative which has been characterized in pharmacological experiments as a centrally acting antihypertensive agent with α-adrenoceptor agonist properties (Scholtysik et al 1975, 1980). Its clinical antihypertensive effectiveness has been clearly established and recently reviewed (Dollery & Jerie 1980; Jerie & Lasance 1981). Apart from its central sympathetic inhibitory actions, two important peripheral effects of guanfacine are the inhibition of the effect of accelerator nerve stimulation in the pithed cat and the reduction of noradrenaline release after nerve stimulation in the rabbit isolated heart (Pacha et al 1975). These effects may contribute to the heart rate lowering effect of guanfacine.

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In view of these findings, it was decided to investigate the effect of endralazine in combination with guanfacine in the spontaneously hypertensive rat and the normotensive dog, the hypothesis being that guanfacine would antagonize the reflex tachycardia induced by endralazine. As the present results demonstrate, guanfacine does in fact inhibit the endralazine-induced tachycardia in both species without any attenuation of the antihypertensive effect of endralazine in the spontaneously hypertensive rat.

MATERIALS AND METHODS

Spontaneously hypertensive conscious rat

The experiments were carried out in conscious male spontaneously hypertensive rats of strain CM-KFMaJ-KYO 1b, mean weight 267 g (210–380 g). The compounds were administered either intravenously or orally. The intravenous doses were given via a catheter implanted in the jugular vein, and the oral doses via a stomach tube. Various dose ratios of endralazine to guanfacine were used and the animals were divided into the following four groups:

(1) Controls (solvent only), (2) endralazine, (3) guanfacine, (4) endralazine + guanfacine. Details of the dosages and routes of administration are given below in the results section and the figures.

For blood pressure measurements, direct and

indirect methods were used. The direct measurement on unrestrained animals was performed according to the method of Weeks & Jones (1960) and was employed for the experiments with intravenous administration of the drugs. Blood pressure and heart rate were recorded directly via a catheter implanted in the aorta under ether anaesthesia at least 2 days before the experiment. For the indirect blood pressure determination used in the experiments with oral administration of the drugs, the animals were immobilized in plexiglass tubes and kept warm in a box maintained at a constant temperature of 32 °C. Systolic blood pressure was measured at the root of the tail, using a sphygmomanometer with a crystal microphone placed distally to it (Friedman & Freed 1949; Gerold & Tschirky 1968). The heart rate was measured electronically via the blood pressure recorder.

The results for blood pressure and heart rate are expressed as changes in pretreatment values. The significance values (P) shown in the figures were calculated using Student's t-test; differences between the effect of endralazine alone and that of combining endralazine with guanfacine were determined.

Normotensive conscious dog

The experiments were carried out on normotensive conscious dogs (3 beagles and 1 Swiss breed beagle-type dog). Three of these animals had been subjected to unilateral nephrectomy in previous experiments for other purposes. The animals were given 60-min infusions of the following substances or saline solutions into the brachial vein: endralazine (0·3 mg kg^{-1} i.v.) followed by 0·9% NaCl; 0·9% NaCl followed by guanfacine (0·2 mg kg^{-1} i.v.); endralazine (0·3 mg kg^{-1} i.v.) followed by guanfacine (0·2 mg kg^{-1} i.v.).

The blood pressure was measured continuously via a Teflon catheter inserted in a carotid loop. Mean arterial blood pressure was measured via a Statham pressure transducer (P 23 DC) and recorded on a 2-channel W + W recorder. The second channel on the recorder was used for recording heart rate, which was triggered by the pulse wave signal.

RESULTS

The effects of endralazine in combination with guanfacine on heart rate and blood pressure in conscious spontaneously hypertensive rats

Intravenous administration

Endralazine, 1 mg kg^{-1}, caused a highly significant fall in blood pressure of 77 ± 9 mmHg within 15 min, starting from a value of 182 ± 9 mmHg; this was followed by a gradual rise, but after 3 h, blood pressure was still 45 ± 6 mmHg ($P < 0·0005$) below the starting value (Fig. 1). Heart rate had risen after 5 min from 422 ± 18 to 543 ± 13 beats min^{-1} (+29%; $P < 0·0005$). It then fell again progressively to 460 ± 19 beats min^{-1} after 3 h (+9%; $P < 0·05$).

Guanfacine, 1 mg kg^{-1}, caused an initial rise in blood pressure from 186 ± 9 to 218 ± 19 mmHg ($P < 0·05$), lasting less than 15 min. Over the next 3 h, blood pressure fell by 28 ± 14 mmHg ($P < 0·05$). Five min after administration of the drug, marked bradycardia was observed with a heart rate of 301 ± 25 beats min^{-1} compared with 417 ± 26 beats min^{-1} initially ($P < 0·0005$). The heart rate then began to climb again, but 3 h after administration, it was still 28 ± 22 beats min^{-1} lower than the pretreatment values (Fig. 1).

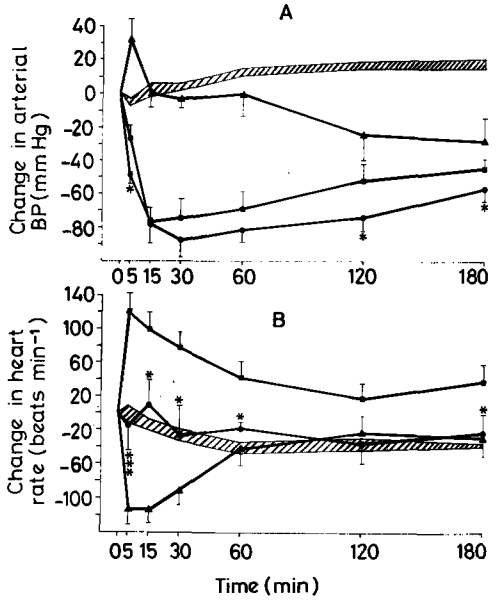

FIG. 1. The effects of endralazine (■; 1 mg kg^{-1} i.v., n = 5, 182 ± 9 mmHg, 422 ± 18 beats min^{-1}) and guanfacine (▲; 1 mg kg^{-1} i.v., n = 5, 186 ± 9 mmHg, 417 ± 26 beats min^{-1}) alone and in combination on blood pressure (A) and heart rate (B) in spontaneously hypertensive rats. The values in brackets are pretreatment values for blood pressure and heart rate for each group (mean ± s.e.). Hatched area, controls (n = 5) (172 ± 5 mmHg; 422 ± 12 beats min^{-1}). ● Guanfacine and endralazine (1 mg kg^{-1} i.v.) n = 6, 182 ± 8 mmHg, 409 ± 9 beats min^{-1}). * $P < 0·05$, *** $P < 0·005$ (against endralazine).

With the combination, the antihypertensive effect between 30 min and 3 h after administration was greater than that observed with either compound on its own. This superiority was statistically significant

($P < 0{\cdot}05$) at 2 and 3 h by comparison with endralazine alone. The heart rate after administration of the combination was similar to controls (Fig. 1). This indicates that the combination did not show the marked tachycardic effect of endralazine or the marked bradycardic effect of guanfacine.

Oral administration

In these experiments, 0·5 mg kg^{-1} endralazine was administered orally together with 0·05, 0·1 or 0·5 mg kg^{-1} guanfacine, and 1 mg kg^{-1} endralazine was combined with 0·2 or 1 mg kg^{-1} guanfacine (Fig. 2).

Guanfacine, 0·05 to 0·5 mg kg^{-1}, inhibited the tachycardia elicited by 0·5 mg kg^{-1} endralazine 1 h after administration almost dose-dependently. Compared with the control values, the tachycardia induced by 0·5 mg kg^{-1} endralazine was completely blocked by 0·5 mg kg^{-1} guanfacine. Guanfacine, 0·2 and 1 mg kg^{-1}, also led to a dose-dependent and significant inhibition of the tachycardic reaction caused by 1 mg kg^{-1} endralazine 1 h after administration. Similar results were obtained 3 and 6 h after administration.

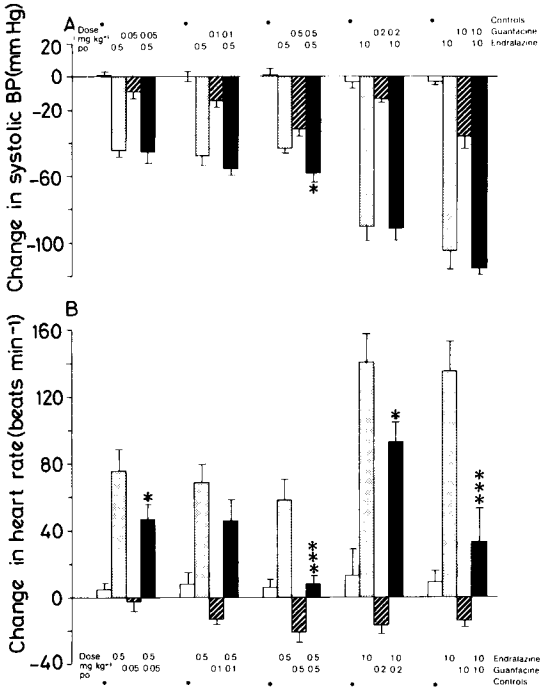

FIG. 2. The effects of endralazine and guanfacine alone and in combination on blood pressure (A) and heart rate (B) in spontaneously hypertensive rats 1 h after oral administration. The pretreatment values for systolic blood pressure and heart rate in the five dose groups were (mean ± s.e.):

	mmHg	beats min^{-1}
Controls (open columns)	217 ± 7 to 225 ± 6	291 ± 5 to 302 ± 7
Endralazine (stippled)	217 ± 7 to 225 ± 6	292 ± 4 to 299 ± 8
Guanfacine (hatched)	216 ± 6 to 225 ± 7	282 ± 4 to 311 ± 11
Endralazine + guanfacine (solid)	217 ± 6 to 224 ± 5	295 ± 4 to 307 ± 6

n = 10 animals per dose; * $P < 0{\cdot}05$, *** $P < 0{\cdot}005$ (against endralazine).

With regard to the antihypertensive effect, the following results were obtained. Endralazine lowered blood pressure 1 h after the administration of 0·5 mg kg^{-1} in three groups of animals by 43 ± 3, 44 ± 4 and 47 ± 6 mmHg systolic blood pressure (see Fig. 2). The pretreatment values were 217 ± 7, 220 ± 5 and 223 ± 7 mmHg. At 1 mg kg^{-1}, endralazine decreased blood pressure in two groups of animals by 90 ± 8 and 104 ± 11 mmHg, respectively (starting values 221 ± 6 mmHg and 225 ± 6 mmHg). Three and 6 h after the administration of the substance, the blood pressure-lowering effect was smaller than that after 1 h.

Guanfacine, in doses between 0·05 and 0·5 mg kg^{-1}, caused a dose-dependent decrease in blood pressure of 9 ± 4 to 31 ± 4 mmHg and after 0·2 and 1 mg kg^{-1} of 13 ± 3 and 36 ± 7 mmHg. The pretreatment values of these five groups of animals were between 216 ± 6 and 225 ± 7 mmHg. The hypotensive activity of guanfacine was diminished within 6 h.

Following the combined administration of endralazine and guanfacine, the antihypertensive effect was slightly greater than that of endralazine alone (Fig. 2). This difference was statistically significant for the combined administration of 0·5 mg kg^{-1} endralazine and 0·5 mg kg^{-1} guanfacine (−58 ± 5 mmHg; $P < 0{\cdot}05$).

The effects of endralazine in combination with guanfacine on heart rate and blood pressure in normotensive conscious dogs

Endralazine given alone at a dose of 0·3 mg kg^{-1} i.v. (as a 60 min infusion) caused a slight fall in blood pressure (−13 ± 11 mmHg) and a marked tachycardia (+108 ± 28 beats min^{-1}). The starting values before the beginning of the infusion were 105 ± 4 mmHg mean blood pressure and 68 ± 8 beats min^{-1}. The fall in blood pressure and tachycardia persisted for 3 h after the end of the infusion. Infusion of a physiological salt solution for 1 h, instead of a guanfacine infusion, did not cause any appreciable changes in the variables measured.

Guanfacine at a dose of 0·2 mg kg^{-1} i.v. (as a 60 min infusion) caused a moderate rise in blood

pressure (+43 ± 13 mmHg) and marked bradycardia (−47 ± 4 beats min^{-1}). The starting values at the beginning of the experiment were 112 ± 4 mmHg and 74 ± 5 beats min^{-1}. After the end of the infusion of guanfacine, the elevated blood pressure returned to the initial value, while the bradycardia persisted in a slightly reduced form (−24 ± 3 beats min^{-1}) for 2 h. A 60 min infusion of a physiological salt solution, which was given instead of endralazine before administration of guanfacine, did not cause any relevant changes in blood pressure or heart rate.

In the combined experiment, endralazine at a dose of 0·3 mg kg^{-1} i.v. caused a slight fall in blood pressure (−17 ± 9 mmHg) and marked tachycardia (+133 ± 19 beats min^{-1}). As may be seen in Fig. 3, the subsequent infusion of 0·2 mg kg^{-1} guanfacine i.v. caused a slight rise in blood pressure (+19 ± 3 mmHg) over the starting value and arrested the sustained tachycardia induced by endralazine. The following values at the beginning of the experiment were measured: 112 ± 7 mmHg mean arterial blood pressure and 59 ± 4 beats min^{-1}.

DISCUSSION

Interaction on heart rate

The antihypertensive drug endralazine is a peripheral vasodilator causing reflex tachycardia in response to blood pressure decrease (Salzmann et al 1979), which is typical for this class of substances. The effects of endralazine are confirmed in the present experiments in spontaneously hypertensive rats and normotensive dogs. Guanfacine is a centrally acting antihypertensive agent with α-adrenoceptor stimulant properties causing long-lasting decrease in heart rate (Scholtysik et al 1975). The present investigation was primarily performed to investigate whether the undesired tachycardic effect of endralazine could be prevented by guanfacine.

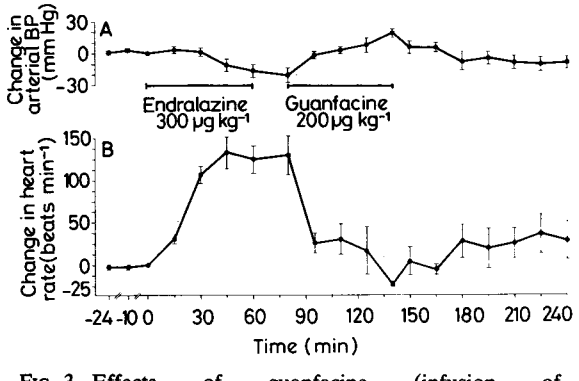

FIG. 3. Effects of guanfacine (infusion of 0·2 mg kg^{-1} h^{-1} i.v.) on the fall in blood pressure and tachycardia caused by endralazine (infusion of 0·3 mg kg^{-1} h^{-1} i.v.) in conscious dogs (n = 4). The starting values at the beginning of the infusion of endralazine were 112 ± 7 mmHg mean blood pressure and 59 ± 4 beats min^{-1}. A, blood pressure. B, heart rate.

As the results show, guanfacine was able to antagonize the effects of endralazine on heart rate over a relatively wide dose-range and at dose ratios of 1:1 up to 10:1 (endralazine:guanfacine) when both drugs were given orally to spontaneously hypertensive rats. After intravenous administration, the tachycardia caused by endralazine was completely prevented by guanfacine in spontaneously hypertensive rats. A similar pattern was seen in normotensive dogs in which guanfacine reversed the established tachycardia induced by endralazine when both drugs were infused intravenously. This means that in rats and dogs, the effects of the two compounds on heart rate balanced out with the result that neither tachycardia nor bradycardia occurred.

The mechanism by which guanfacine antagonizes the effect of endralazine on heart rate might be peripheral as well as central. The evidence for a peripheral site of action is that guanfacine inhibits the effects of accelerator nerve stimulation in cats (Pacha et al 1975; Scholtysik et al 1975), an observation which is supported by the finding that in the rabbit isolated heart, guanfacine inhibits noradrenaline release due to nerve stimulation by activating presynaptic α-adrenoceptors (Pacha et al 1975). This inhibitory effect of guanfacine on peripheral cardiac adrenergic neurons could explain why it reduces the endralazine-induced tachycardia. On the other hand, it is possible that the central sympathoinhibitory action of guanfacine described by Scholtysik et al (1975) is responsible for the inhibition of the reflex tachycardia caused by endralazine. Furthermore, guanfacine markedly enhances the vagally mediated reflex bradycardia induced by baroreceptor activation (Scholtysik et al 1980). Thus, increased vagal tone by a central action of guanfacine could contribute to the antagonism of the endralazine-induced tachycardia.

Interaction on blood pressure

The interaction between endralazine and guanfacine on blood pressure is not as clear as the interaction on heart rate. The combination of both drugs in spontaneously hypertensive rats after intravenous administration caused a greater fall in mean arterial blood pressure than either of the two compounds on its own. Thus, by combining the two drugs, blood pressure is lowered more effectively than by either of

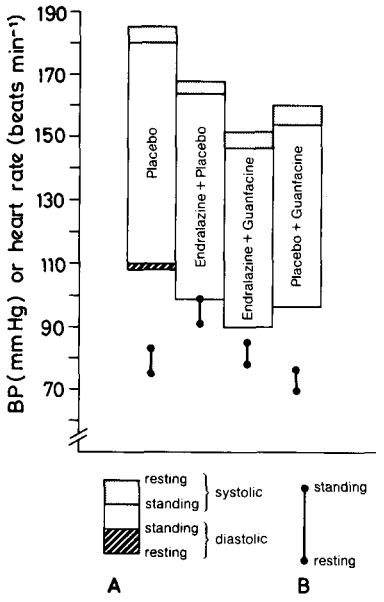

FIG. 4. Blood pressure (A) and heart rate (B) before and during treatment with endralazine and guanfacine alone and in combination in 33 hypertensive patients. The following doses were used: endralazine 3 × 6·99 mg + guanfacine 3 × 0·67 mg. Values communicated with kind permission of Prof. C. Hitzenberger.

them alone. After oral administration, only a tendency to increased antihypertensive efficacy was seen in the combination. However, it is worth noting that there was one combination where there was a significant enhancement. That was with the smallest dose of endralazine and the larger dose of guanfacine.

The reason for this hardly additive antihypertensive effect of the combination in spontaneously hypertensive rats is not known. A possible explanation might be that the strong antihypertensive effect of the relatively high doses of endralazine is difficult to enhance by addition of a second antihypertensive agent. Another explanation could be that guanfacine, which has α-adrenoceptor stimulant properties, can easily constrict resistance vessels in the rat which have been previously relaxed by endralazine. Therefore, further decrease in peripheral resistance due to the administration of endralazine, and consequently further decrease in blood pressure, can hardly be achieved by guanfacine.

In the experiments with normotensive dogs, blood pressure was decreased by endralazine. Subsequent infusion of guanfacine reversed the vasodepressor effect of endralazine. This is not surprising since it has been shown that guanfacine induced a strong vasoconstrictor effect in normotensive dogs after intravenous administration (Scholtysik et al 1975). It should be emphasized that in the present experiments, guanfacine caused an increase in blood pressure only during the infusion. After stop of the infusion, blood pressure declined rapidly to a level slightly below the pretreatment values.

General conclusion

The present results in conscious spontaneously hypertensive rats and normotensive dogs have demonstrated the advantage of the combination of endralazine and guanfacine over endralazine alone. In both species, guanfacine inhibited the tachycardia which is an undesirable side-effect of treatment with peripheral vasodilators. As far as heart rate is concerned, our experimental results are fully confirmed by clinical observations (Fig. 4). Hitzenberger & Stumpe (1980) reported an inhibition of the endralazine-induced tachycardia by addition of guanfacine when applied in a dose-ratio of 10:1 (endralazine:guanfacine). As far as blood pressure is concerned, the clinical results are much more favourable than expected from our results in spontaneously hypertensive rats. The antihypertensive effect was greater than the effects of the single components (Hitzenberger & Stumpe 1980). It is not possible to decide whether differences in the sensitivity to the vasoconstrictor effect of guanfacine are responsible for the differences between rats and men with respect to the antihypertensive effectiveness of the combination.

However, the presented results offer a new rational approach for combined antihypertensive treatment.

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