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The effects of treatment and of withdrawal of treatment with guanfacine and clonidine on blood pressure and heart rate in normotensive and renal hypertensive rats

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Disagreement in the literature about the occurrence of rebound hypertension (hypertensive overshoot) in animal models initiated this investigation. Oral doses of clonidine (0·03 mg kg^{-1}) or guanfacine (0·3 mg kg^{-1}) were administered twice daily during three weeks to groups of normotensive and renal hypertensive rats. Blood pressure and heart rate were measured immediately before and 3 h after the first daily dose, and compared with values obtained from placebo-treated control rats. Treatment with either drug induced large daily fluctuations rather than sustained falls in blood pressure. In the normotensive, but not in the hypertensive groups, the morning blood pressures measured before the first daily dose were significantly elevated over those of the control groups after 9 and 5 days of treatment with clonidine or guanfacine. This elevation persisted for 3 days after drug withdrawal. It is concluded that in the rat the duration of action of both drugs was short, so that twice daily administration of both drugs similarly produced large daily fluctuations rather than sustained falls in blood pressure. Blood pressure rises developed during treatment rather than after withdrawal in normotensive rats only. Therefore, this type of study does not relate well to the human situation and different experimental approaches to this problem are needed.

Withdrawal of clonidine therapy in hypertensive patients can lead to a 'rebound hypertension' which may include a hypertensive overshoot of critical magnitude, tachycardia, anxiety, nervousness, restlessness and in some instances headache and vomiting (withdrawal syndrome) (Hökfelt et al 1970; Hansson et al 1973). Animal experiments to investigate this phenomenon have produced contradictory results.

In experiments in normotensive rats, Oates et al (1977, 1978) and Prop (1978) found an exaggerated increase in blood pressure following cessation of two to three weeks treatment with clonidine while Cavero et al (1977) and Dix & Johnson (1977) did not detect any overshoot reaction in blood pressure. Oates et al (1978) reported that after three weeks treatment with guanfacine (BS 100–141; *N*-amidino-2-(2,6-dichloro-phenyl)acetamide hydrochloride) in normotensive rats a marked hypertensive overshoot occurred after withdrawal which was greater than that seen after clonidine. This report is at variance with clinical observations which have shown that guanfacine, in contrast to clonidine, induces only a slight withdrawal syndrome or none at all (Turner 1974; Jäättelä 1976; Kirch & Distler 1978).

In hypertensive rats Cavero et al (1977) and Prop (1978) found no 'rebound hypertension' following withdrawal of clonidine treatment. No information is available on the effects of guanfacine withdrawal in hypertensive rats.

The experiments reported here were carried out to investigate the influence of three weeks treatment with guanfacine and clonidine on the blood pressure and heart rate of normotensive and hypertensive rats and the effects on these two parameters of withdrawal of drug treatment. The aim of the investigation was to test the suitability of the mode of oral administration twice daily to demonstrate a rebound phenomenon, i.e. an overshoot of blood pressure and/or heart rate above pretreatment levels or over the values at the end of the treatment period.

METHODS

Animals and experimental design

Experiments were made on 42 female albino rats (SIV 50 strain from Ivanovas, Kisslegg, W. Germany). In half the animals renal hypertension was induced according to Grollman (1944). At five weeks of age the animals were anaesthetized with ether and a silk figure-of-eight ligature tied around one kidney. One week later the contralateral kidney was removed in a second operation. The animals were used for experiment at ten weeks of age.

During the three week treatment period drugs were administered twice daily (morning and evening) using a stomach tube. The normotensive and hyper-

tensive animals were randomly divided into groups of 7 and each group treated with one of the following: guanfacine 0·3 mg kg^{-1} orally twice daily; clonidine 0·03 mg kg^{-1} orally twice daily; demineralized water 10 ml kg^{-1} orally twice daily (controls).

For blood pressure determinations the animals were placed in an environment maintained at 34 °C. The systolic blood pressure was measured from the tail using a sphygmomanometer cuff and a crystal microphone placed distally to it (Friedman & Freed 1949; Geddes 1970). Heart rate was derived electronically from the microphone signal. Control blood pressure measurements were made twice daily, once in the morning and again 6–7 h later in the afternoon on the two days before the experiment was begun. During the three week treatment period blood pressure and heart rate were measured twice weekly; immediately before and 3 h after drug administration on one day and immediately before drug administration on the following day (i.e. 16–17 h after the second dose of the previous day). After stopping treatment, measurements were made in the morning (t_0), at t_0 + 3 h and at t_0 + 7 h on three successive days immediately after withdrawal (days 24–26) and again on the 29th, 31st and 38th days of the experiment. The experiment was terminated 14 days after withdrawal of drug treatment.

Statistical analysis

Time-dependent differences within groups were analysed using a paired difference *t*-test.

Because of a skew distribution of the values obtained, it was not possible to perform an analysis of variance to compare groups. Therefore, the absolute values obtained during the analysis period (days 3–23 and 24–38) were added together to obtain a value for each animal and these values were analysed using the Kruskal–Wallis test and following the multiple comparison test by Dunn–Bonferroni (Hollander & Wolfe 1973).

The values for blood pressure and heart rate presented in the results are the means ± standard errors of the means (s.e.) obtained from each group.

RESULTS

(a) *Normotensive rats*

During the three week treatment period blood pressure and heart rate were reproducibly and mostly significantly reduced when measured 3 h after drug administration (Fig. 1). The reduction in blood pressure was between 13 ± 2 and 29 ± 3 mm Hg ($P < 0{\cdot}005$ to $0{\cdot}0005$) in the clonidine-treated animals and 10 ± 2 and 23 ± 4 mm Hg ($P < 0{\cdot}025$ to $0{\cdot}0005$) in those receiving guanfacine. From day 5 on, the blood pressures of the guanfacine-treated animals were usually significantly higher in the recovery phase (i.e. immediately before each daily drug administration) than in the pre-drug control period (2 $P < 0{\cdot}025$ to $0{\cdot}005$) (Fig. 1). Similarly, the blood pressures of the clonidine-treated rats, measured between days 9 and 23, were significantly

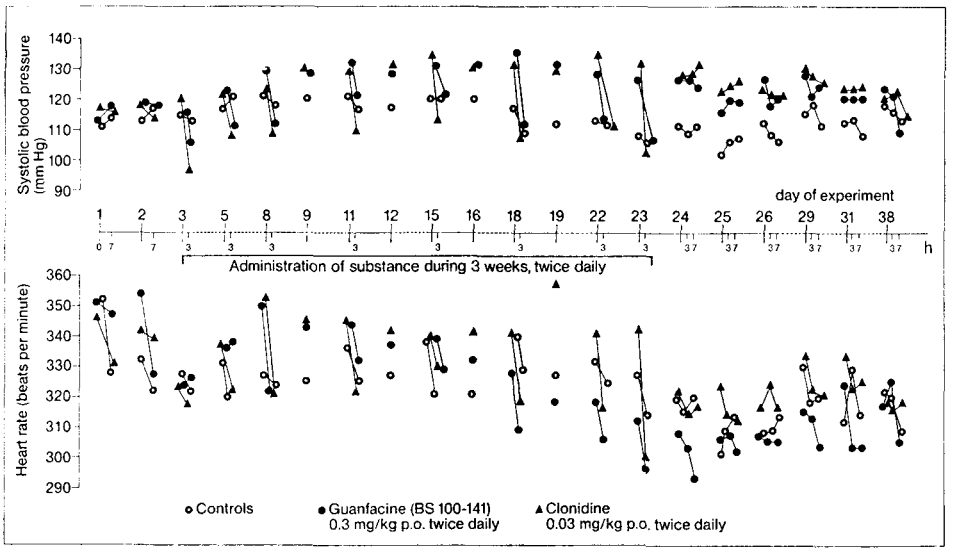

FIG. 1. The effects of guanfacine (BS 100–141) and clonidine in the conscious, normotensive rat. Oral doses of clonidine (0·03 mg kg^{-1}) or guanfacine (0·3 mg kg^{-1}) were administered twice daily during three weeks. The influence of these drugs on blood pressure and heart rate during treatment and after withdrawal of drug treatment is shown.

higher than those obtained in the control period (2 $P < 0{\cdot}02$ to $0{\cdot}0005$) (Fig. 1). A comparison between the groups revealed that systolic blood pressure (measured before each first daily drug administration) of the clonidine- and guanfacine-treated animals during the drug treatment period (days 3–23) was significantly higher than that of the controls (2 $P < 0{\cdot}01$ and 2 $P < 0{\cdot}05$).

On the day after withdrawing treatment (day 24, Fig. 1) the mean blood pressures of the guanfacine- and clonidine-treated animals, measured in the morning, remained significantly higher than those of the controls (2 $P < 0{\cdot}05$ and $< 0{\cdot}01$ respectively). Three and 7 h later as well as on the second day following drug withdrawal, mean blood pressures in the guanfacine and control groups were not significantly different from each other, whereas mean blood pressure in the clonidine-treated animals was significantly higher than that of the controls (2 $P < 0{\cdot}05$ to $0{\cdot}01$). On the third day (day 26) the blood pressures of both the clonidine- and the guanfacine-treated groups were significantly higher than those of the control animals (2 $P < 0{\cdot}05$). Subsequently, the blood pressures of the guanfacine- and clonidine-treated animals fell slightly while those of the controls increased somewhat and no further statistically significant differences were detected.

As with systolic blood pressure, heart rate measured 3 h after the daily drug administration was markedly reduced, but several of the control animals also showed quite marked bradycardia. On the first day after stopping treatment (day 24) the mean heart rate of the guanfacine-treated group was significantly lower than that of the control animals (2 $P < 0{\cdot}05$). There was no significant difference between the mean heart rates of the clonidine-treated and control groups.

All animals survived the experiment and no side effects were noted.

(b) *Hypertensive rats*

Reductions in blood pressure and heart rate were observed 3 h after daily administration of either guanfacine or clonidine (Fig. 2). Guanfacine reduced the blood pressure by between 10 ± 12 and 36 ± 14 mm Hg and clonidine by between 11 ± 12 and 42 ± 17 mm Hg. Statistically significant hypotensive effects were obtained with guanfacine on days 3, 8, 11, 18, 22 and 23 ($P < 0{\cdot}025$ to $< 0{\cdot}0005$) and with clonidine on days 3, 8, 15, 18 and 23 ($P < 0{\cdot}025$ to $< 0{\cdot}001$). With the exception of this difference no other significant differences were found between the blood pressures of the guanfacine- or clonidine-treated animals and those of the controls either during drug treatment or after withdrawal.

Systolic blood pressure gradually increased during the three week treatment period in the control group and in the guanfacine- and clonidine-treated groups (Fig. 2). In several cases blood pressure measurements before each daily drug administration showed significantly higher values ($P < 0{\cdot}05$ to $< 0{\cdot}005$) compared with pretreatment values. On most days after withdrawal of treatment mean blood pressures of the control group and the guanfacine- and clonidine-treated groups were statistically significantly higher ($P < 0{\cdot}05$ to $< 0{\cdot}005$) than during the pretreatment period. However, there was no statistically significant difference between the three groups.

The heart rate was reduced 3 h after treatment with either clonidine or guanfacine, the effects of clonidine being more marked. After withdrawal of drug treatment there were no statistically significant differences.

In the control group of hypertensive rats one animal died on the last day of the experiment. In the clonidine-treated group 3 out of 7 animals died (on the 11th, 18th and 23rd days of the experiment respectively). Side effects included piloerection, exophthalmos and sedation. Three animals in the guanfacine-treated group also died, but they survived somewhat longer than those which died in the clonidine-treated group (15th, 25th and 29th days respectively). Side effects included sedation and exophthalmos; in contrast to the clonidine-treated animals, no piloerection was observed.

DISCUSSION

In these experiments in normotensive and renal hypertensive rats administration of clonidine (0·03 mg kg^{-1} orally twice daily) or guanfacine (0·3 mg kg^{-1} orally twice daily) during three weeks led to marked, reproducible and statistically significant reductions in systolic blood pressure which were accompanied by bradycardia. Both effects were relatively short-lasting, complete recovery had occurred within 17 h (Fig. 1 and 2). Using these doses, it was not possible to produce a sustained fall in blood pressure. With guanfacine this can be explained by the fact that the drug is more completely metabolized in the rat than in man (Kiechel, personal communication). Scholtysik et al (1975) found that after administration of guanfacine (2 mg kg^{-1} orally) in the normotensive rat the hypotensive effect was maximal at 2 h and blood pressure had almost returned to normal within 6 h. These authors also reported that in

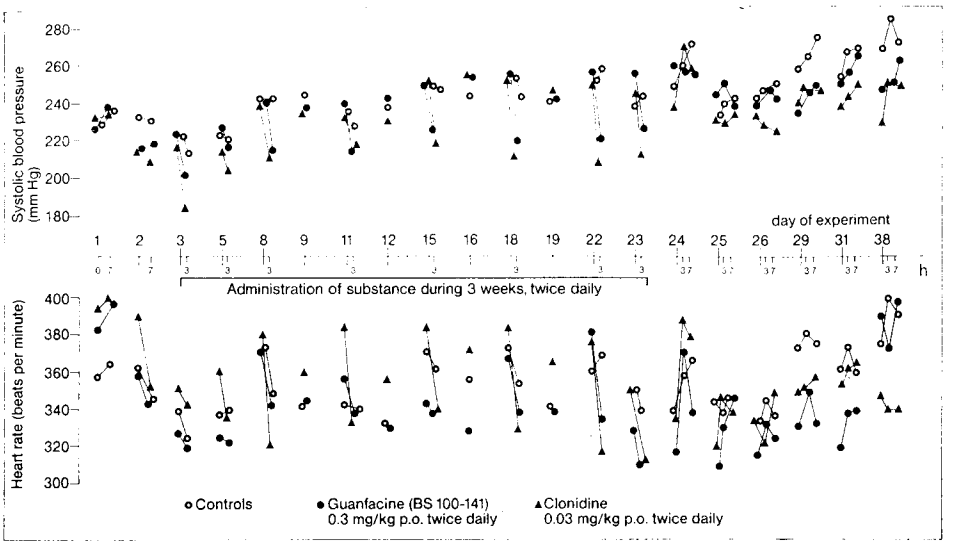

FIG. 2. The effects of guanfacine (BS 100–141) and clonidine in the conscious, renal hypertensive Grollman-rat. Oral doses of clonidine (0·03 mg kg^{-1}) or guanfacine (0·3 mg kg^{-1}) were administered twice daily during three weeks. The influence of these drugs on blood pressure and heart rate during treatment and after withdrawal of drug treatment is shown.

DOCA hypertensive rats the maximum hypotensive effect of guanfacine was attained after 4 h and thereafter blood pressure gradually returned to hypertensive levels. This observation of a short-lasting blood pressure lowering effect could be relevant for the interpretation and extrapolation of results obtained from rats to man. In the treatment of hypertension continuous blood pressure control is usually achieved. The blood pressure measurements in rats under treatment revealed increasing daily fluctuations of blood pressure, a situation which is avoided in patients.

In normotensive rats, during three weeks treatment with either guanfacine or clonidine blood pressure measured immediately before the first daily dose of the drug increased progressively from day 5 until it was significantly higher than that of the untreated controls and than that of the pretreatment period (Fig. 1). This effect could be interpreted as a hypertensive overshoot. The elevated blood pressure persisted after withdrawal of both drugs, and the increases in blood pressure in the clonidine- and guanfacine-treated animals were of similar magnitude on the first day after withdrawal of treatment (16–20 and 13–17 mm Hg respectively).

Oates et al (1978) reported that 25 h after clonidine withdrawal in the normotensive rat the mean systolic blood pressure was 18 mm Hg higher than that of control animals. This is in good agreement with our own results since, at the 7 h measurement on day 24 (i.e. 25 h after the last dose of clonidine) we found that the blood pressure of the treated animals was 20 mm Hg higher than that of the controls. However, whereas in our experiments guanfacine withdrawal induced increases of 13–17 mm Hg above control values (i.e. similar to the increases seen after clonidine withdrawal), Oates et al (1978) reported increases of 57 mm Hg at 25 h and 42 mm Hg at 30 h after guanfacine withdrawal. Thus, quantitative differences exist between our results and those of Oates et al (1978). Unfortunately, Oates et al do not provide any information concerning the blood pressures or heart rates of their animals either before or during the period of drug treatment. Furthermore, in contrast to our experiments in which conscious rats were employed, Oates et al (1978) used anaesthetized animals, so that a direct comparison of results is probably not valid. In normotensive conscious rats, Prop (1978) found an increase in blood pressure after clonidine withdrawal whereas Cavero et al (1977) and Dix & Johnson (1977) could not observe any hypertensive overshoot, although the latter authors did report an increase in heart rate following withdrawal.

In hypertensive rats, with the exception of the reductions in blood pressure and heart rate seen 3 h after drug administration, we were unable to detect any significant differences between the blood press-

ures of drug treated and control animals. Although blood pressures of the guanfacine- and clonidine-treated groups were statistically significantly higher than the pretreatment values, this result cannot be interpreted as a hypertensive overshoot reaction because blood pressure of the control group showed an increase of similar magnitude and there was no difference in height of blood pressure between the three groups. In the case of clonidine these results are in agreement with those of other authors. Cavero et al (1977), in experiments using spontaneously hypertensive rats treated for two weeks with clonidine (0·2 mg kg^{-1} s.c., twice daily), did not detect any hypertensive overshoot. Similarly, Prop (1978) was unable to demonstrate any increase of blood pressure after withdrawal of clonidine in the same model. However, renal hypertensive cats and renal hypertensive mongrel dogs (but not beagles) did show rebound increases in blood pressure (Cavero et al 1977).

In summary, it is concluded that in the rat the duration of action of guanfacine and clonidine was short, so that twice daily administration of both drugs during three weeks similarly produced large daily fluctuations rather than sustained falls in blood pressure. Blood pressure rises developed during treatment rather than after withdrawal in normotensive rats only. The increases in blood pressure were of similar magnitude in the guanfacine- and clonidine-treated animals. These findings are in contrast to those of Oates et al (1978) who found that guanfacine showed a bigger increase of blood pressure after withdrawing the substance than clonidine in anaesthetized normotensive rats. In renal hypertensive rats neither guanfacine nor clonidine treatment led to any detectable overshoot reaction in blood pressure compared with control animals. Therefore this type of study does not relate well to the human situation and different experimental approaches to this problem are needed.

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