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Withdrawal syndrome after continuous infusion of clonidine in the normotensive rat

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The effects on heart rate and arterial pressure of continuous subcutaneous infusion of clonidine (500 μg kg^{-1} day^{-1}) via an ALZET miniosmopump and sudden termination of this treatment were studied in conscious normotensive rats. No consistent decreases of heart rate and blood pressure were observed during the clonidine infusion. Within a few hours following removal of the minipumps, a pronounced and long-lasting tachycardia developed. Mean arterial pressure did not exceed control level consistently, but transient blood pressure 'upswings' were observed. The withdrawal phenomena were accompanied by a small, significant rise in plasma noradrenaline content. During the clonidine-infusion period piloerection and sedation were prominent. The present study indicates, that the occurrence of the withdrawal phenomena following cessation of clonidine treatment is not dependent upon a decrease of blood pressure and heart rate preceding clonidine withdrawal. Furthermore, the model presented may be suitable for the study of withdrawal phenomena of other antihypertensive drugs.

The abrupt cessation of clonidine treatment in hypertensive patients can provoke symptoms of sympathetic overactivity, like rises in blood pressure, cardiac frequency and plasma noradrenaline concentration (Hoobler & Kashima 1977; Weber 1980). These changes only seem to occur in patients suffering from serious hypertension and subjected to prolonged clonidine treatment (Chrysant & Whitsett 1978).

It would be of interest to develop an animal model in order to study the withdrawal phenomena, not only for clonidine but also for other potential antihypertensive drugs. However, it has been very difficult to reproduce this phenomenon in animals, and the results so far are controversial (Prop 1978; Salzmann 1979; Oates et al 1978; Finch & Hicks 1980; DiStefano et al 1980). This is probably due to the difficulties in maintaining a sufficiently high and reasonably constant blood concentration of clonidine as a result of the drug's short half-life (Jarrott & Spector 1978).

Recently, we have attempted to overcome this problem by using the ALZET osmotic minipump, which allows a continuous infusion of clonidine over a period of almost two weeks. Interruption of this infusion of clonidine in spontaneously hypertensive rats led to a genuine overshoot in heart rate. Arterial pressure did not exceed pretreatment levels, but typical transient 'upswings' were observed (Thoolen et al 1981).

It has been suggested, that the clonidine withdrawal phenomenon would be more pronounced in the normotensive than in the hypertensive rat (Prop 1978; Salzmann 1979). For this reason, we have investigated the effects on blood pressure and heart rate of continuous infusion and sudden withdrawal of clonidine in conscious, unrestrained normotensive rats. Since little information is available about the exact onset and time course of the clonidine withdrawal phenomenon in the rat, special attention has been paid to this subject.

MATERIALS AND METHODS

Fourty male normotensive Wistar rats (250–270 g), raised in our laboratory, were used. In 20 animals an ALZET osmotic minipump (model 1702, lot 02701; Alza Corporation, Palo Alto, Cal. U.S.A.), containing clonidine hydrochloride (Boehringer Ingelheim, F.R. Germany), dissolved in 0·9% NaCl (saline), was implanted subcutaneously in the dorsal region via a small skin incision under hexobarbitone-sodium anaesthesia (150 mg kg^{-1} i.p.). Through this pump a continuous infusion of 500 μg kg^{-1} day^{-1} was effected. The remaining 20 animals received saline-charged minipumps. The nominal pumping rate of the devices was 0·63 μl h^{-1}. This guaranteed a constant flow during at least 12 days in vivo in rat

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subcutaneous tissue (product information, Alza Corp.). At the end of the twelfth day of infusion the minipumps were removed from the conscious animals through the skin incision after cutting the silk suture. Blood pressure and heart rate were recorded directly via indwelling abdominal aortic PE-10 catheters, inserted under hexobarbitone-sodium anaesthesia (150 mg kg^{-1} i.p.), according to the method of Still & Whitcomb (1956) as modified by Weeks & Jones (1960). Generally, arterial pressure and heart rate could be measured during 6–12 days, until obstruction of the catheter occurred. To cover the complete infusion and withdrawal period, subgroups of 4–8 rats were taken, in which catheters were implanted several times during the clonidine/saline infusion. In 5 animals of both groups this intervention was performed two days before insertion of the minipump. Blood pressure and heart rate of these animals were considered pre-infusion values. Only the data obtained two and more days after catheterization were taken into account. During the infusion period blood pressure and heart rate recordings were taken daily between 1500 and 1700 h, using Statham P 23 Db pressure transducers and Hellige HE 19 devices. Directly following the removal of the minipumps arterial pressure and heart rate were continuously monitored for 12 h, and later at appropriate times as indicated. Single datum points for heart rate and mean arterial pressure were calculated at 1-h intervals. Arterial blood samples (0·7 ml) were taken through the aortic catheter at the eleventh day of the infusion period and 12 h after the removal of the minipumps. Plasma noradrenaline content was determined via the radioenzymatic method described by Henry et al (1975). Sedation in clonidine- and saline-treated rats was evaluated at the tenth day of the infusion period by measuring the hexobarbitone-induced loss of righting reflex (150 mg kg^{-1} i.p.). Throughout the experiment the animals were housed in individual macrolon cages. Normal rat chow and tap water were freely available.

All values given represent mean ± s.e.m. Statistical significance was ascertained by a combination of analysis of variance and a *t*-test (Bonferroni method) (Wallenstein et al 1980).

RESULTS

During the 12-day infusion of clonidine (500 μg kg^{-1} day^{-1}) no consistent decreases in heart rate and arterial pressure were observed. In the clonidine-treated rats both cardiac frequency and blood pressure were lower than in the saline-treated group, but

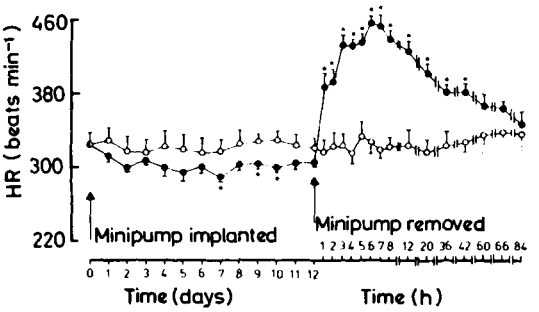

FIG. 1. Heart rate of conscious unrestrained normotensive rats during continuous subcutaneous infusion of clonidine (500 μg kg^{-1} day^{-1}, ●—●) or saline (○—○) and after sudden cessation of treatment. Symbols represent mean ± s.e.m. (n = 7–10):* $P < 0.05$.

significant differences occurred only on certain days (Figs 1, 2). During the infusion period piloerection was prominent in the clonidine-treated animals.

The loss of righting reflex, induced by intraperitoneal injection of 150 mg kg^{-1} hexobarbitone sodium, amounted to 232 ± 29 min (n = 5) in the clonidine-, and 59·3 ± 4·8 min (n = 5) in the saline-treated rats ($P < 0.05$). The animals did not display aggressive behaviour at any time during the experiment.

Following removal of the minipumps from the clonidine-treated rats, heart rate rose rapidly to high values, in contrast to the saline-treated group, in which heart rate remained on the same level as during the infusion period. One hour after the clonidine withdrawal, cardiac frequency had risen from 313 ± 3·7 beats min^{-1} (n = 10) to 389 ± 14·3 beats min^{-1} (n = 10). A maximum of 456 ± 4·9 beats min^{-1} (n = 10) was achieved 6 h after cessation of the clonidine infusion. Heart rate returned to control levels during the subsequent days. Eighty-four hours after removal of the pumps the heart rate of clonidine- and saline-treated rats were no longer significantly different (Fig. 1).

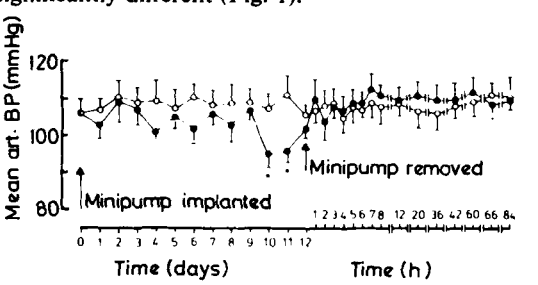

FIG. 2. Mean arterial pressure of conscious unrestrained normotensive rats during continuous subcutaneous infusion of clonidine (500 μg kg^{-1} day^{-1}, ●—●) or saline (○—○) and after sudden cessation of treatment. Symbols represent mean ± s.e.m. (n = 7–10). *: $P < 0.05$

After clonidine withdrawal, mean arterial pressure did not consistently rise above control level (Fig. 2). However, the blood pressure tracings started to show typical 'upswings' (Fig. 3). These 'upswings' were sudden in onset and lasted 3–6 min, during which period blood pressure was raised by 30–50 mm Hg. The number of 'upswings' per hour increased from

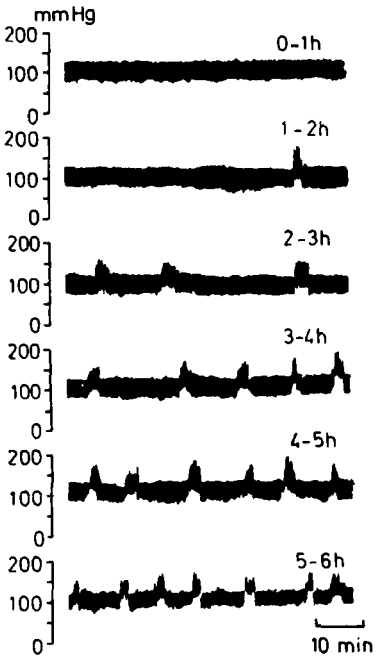

FIG. 3. Arterial pressure recordings of a conscious unrestrained normotensive rat, 0–6 h after sudden termination of a continuous subcutaneous clonidine infusion (500 μg kg^{-1} day^{-1}). Typical experiment. Note the development of blood pressure 'upswings'.

1 h following pump removal onwards, reaching a maximum at 8–10 h (Fig. 4). During the following days, their incidence decreased and the phenomenon had almost dissappeared at 42 h after clonidine withdrawal. 'Downswings' of blood pressure did not occur. After discontinuing the saline infusion in the control animals, no blood pressure 'upswings' were observed.

During the infusion period no significant difference in plasma noradrenaline concentration was found between the clonidine- and saline-treated group. (0·51 ± 0·09 ng ml^{-1}, n = 7, and 0·58 ± 0·11 ng ml^{-1} n = 8, respectively). Twelve hours after cessation of clonidine infusion plasma noradrenaline concentration had increased to 0·81 ng ml^{-1} (n = 7), whereas in the saline-treated controls 0·48 ± 0·07 ng ml^{-1} was found ($P < 0·05$).

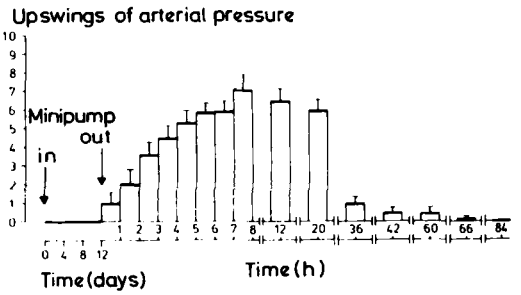

FIG. 4. Incidence of blood pressure 'upswings' following cessation of clonidine infusion in conscious unrestrained normotensive rats. Columns represent mean, vertical lines represent s.e.m. (n = 8).

DISCUSSION

The present investigation in normotensive rats has demonstrated that the withdrawal of clonidine after continuous infusion of 500 μg kg^{-1} day^{-1} over a 12-day period results in the development of pronounced tachycardia and a period of labile blood pressure. These phenomena occurred despite the inefficacy of this dose of clonidine in consistently reducing heart rate and blood pressure in the conscious normotensive rat. Zandberg (1977) has previously demonstrated the inability of clonidine in decreasing blood pressure in conscious normotensive rats. The withdrawal symptoms observed in the present study were very similar to those found in our study in the spontaneously hypertensive rat, in which a sustained and considerable reduction of blood pressure and heart rate was achieved by the same dose of clonidine (Thoolen et al 1981). Therefore, the occurrence of the withdrawal phenomenon, as observed, would not seem to be dependent upon the hypotensive and bradycardic action preceding the cessation of clonidine treatment.

It should be noted, that the tachycardia and the lability of arterial pressure appeared very rapidly after discontinuing the clonidine infusion. The elevation of plasma noradrenaline concentration during the clonidine withdrawal phase suggests that the tachycardia observed may be due to an increased sympathetic output.

Our results show no evidence for a sustained blood pressure overshoot. However, the blood pressure 'upswings', which developed parallel to the tachycardia, might be responsible for the controversy in the literature about the occurrence of a blood pressure rebound after clonidine withdrawal (Prop 1978; Salzmann 1979). Since in these studies mostly indirect blood pressure measurements were per-

formed (tail-cuff method) the lability of blood pressure may not have been obvious. In a recent paper DiStefano et al (1980) demonstrated rebound tachycardia in normotensive rats after cessation of clonidine administration via the drinking water. However, blood pressure upswings were not reported. It is difficult to compare this study to ours, since the methodologies are very different. DiStefano et al demonstrated blood pressure and heart rate decreases in clonidine-treated rats under pentobarbitone anaesthesia and rebound tachycardia in conscious animals. This is in contrast to our experimental design, in which the cardiovascular effects of clonidine infusion as well as sudden withdrawal were studied in conscious, unrestrained rats. Furthermore, the administration of clonidine via the drinking water might not be expected to result in reasonably constant blood levels, since rats drink mainly during night time (personal observation). It is likely, that this procedure results in discontinuous administrations with rather large variations in dose.

Several investigations have demonstrated that treatment with clonidine, including administration via the drinking water, at doses comparable to ours, results in severely aggressive behaviour of the rats (Morpurgo 1968; Laverty & Taylor 1969; Paalzow 1978). No such effect was seen during the continuous administration via the minipumps. In fact, sedation was prominent in the clonidine-treated rats, as was obvious from the prolongation of the loss of righting reflex induced by hexobarbitone. In man the administration of therapeutic doses of clonidine is frequently accompanied by sedation (van Zwieten 1975). In this respect, the use of the osmotic minipump seems to be more relevant to the clinical situation than any other route of administration so far used.

In summary, the present findings indicate that the continuous infusion of clonidine via the ALZET osmotic minipump and subsequent sudden termination of this treatment in the normotensive rat results in the rapid development of severe tachycardia and the occurrence of transient blood pressure 'upswings'. The continuous infusion of clonidine in the dosage used does not result in a consistent reduction of arterial pressure and heart rate. Therefore, neither the existence of severe hypertension before clonidine treatment, nor the consistent reduction of heart rate and blood pressure by clonidine seem to be a prerequisite for the occurrence of clonidine withdrawal symptoms in the rat. Since in man blood pressure is generally measured via indirect, momentary methods it is tempting to speculate that the clonidine discontinuation syndrome in man is also mainly associated with a labile blood pressure, rather than a sustained increase above pre-dose level.

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