J. Pharm. Pharmacol. 1989, 41: 353–355
Communicated October 11, 1988

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# Serum glucose and insulin levels in normotensive (WKY) and spontaneously hypertensive (SH) rats during and after the cessation of continuous (10 day) clonidine infusion

S. J. LEWIS, M. DUNLOP*, B. JARROTT, *University of Melbourne, Clinical Pharmacology and Therapeutics Unit, Austin Hospital, Heidelberg, Victoria 3084, Australia and *Department of Medicine, Royal Melbourne Hospital, Parkville, Victoria, Australia*

**Abstract**—The concentrations of glucose were elevated whereas those of insulin were decreased in the sera of normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive (SH) rats on day 10 of a continuous subcutaneous infusion of clonidine (10 $\mu$g $kg^{-1}$ $h^{-1}$ for 10 days). Fifteen to 18 h after cessation of infusion, the glucose levels of both strains had fallen to below those of the respective controls whereas the insulin levels remained suppressed. As such the hypoglycaemia may be related to the general increase in metabolic requirements associated with the post-infusion 'withdrawal syndrome'.

In normotensive humans, the acute administration of clonidine produces a rise in blood glucose levels whereas chronic therapy with this drug does not produce sustained changes in blood glucose or insulin levels or insulin release in response to ingested glucose in either normotensive or hypertensive subjects (see Boyar et al 1980; Stornello et al 1986). This suggests that tolerance may develop to the effects of clonidine on glucose metabolism in humans. In rats, several of the actions of clonidine appear to diminish during chronic treatment. For example, tolerance develops to the analgesic (Paalzow 1978) and sedative actions (Laverty & Taylor 1969). Consequently, the question arises as to whether the effects of clonidine on serum glucose and insulin concentrations are maintained in the rat during chronic treatment and whether these parameters are disturbed further upon cessation of treatment, especially since the rats display a "withdrawal" syndrome which is characterized by cardiovascular disturbances, hyperthermia and "opiate abstinence-like" phenomena which include head and body shakes, paw tremor and teeth chatter (Thoolen et al 1981; Jarrott et al 1984).

Ishii et al (1985) reported that the usual rise in serum insulin levels in response to glucose (750 mg $kg^{-1}$ i.v.) was attenuated in anaesthetized normotensive rats which had received 10 days of clonidine treatment (administered via the drinking water; 10 $\mu$g $mL^{-1}$) whereas the glucose-induced rise in insulin was enhanced in rats which were withdrawn from the clonidine treatment. As the animals were anaesthetized at the time of examination of glucose metabolism and the treatment regime was not truly chronic, the results must be viewed with circumspection.

In the present study the effects of a continuous 10 day clonidine infuson (10 $\mu$g $kg^{-1}$ $h^{-1}$ via osmotic minipumps), and then the cessation of this infusion, on the glucose and insulin levels of normotensive (Wistar-Kyoto, WKY) and spontaneously hypertensive (SH) rats have been examined. The SH strain was chosen principally because this model of hypertension has many similarities to essential hypertension in humans (see Rapp 1983), and, as such, the results could be relevant to those found in man. The second reason for examining the SH was to find whether peripheral $\alpha$-adrenoceptor function. i.e. that regulating glucose metabolism, is altered in this strain.

Correspondence to: S. J. Lewis, Department of Pharmacology, College of Medicine, 2-272 Bowen Sciences Building, University of Iowa, Iowa City, Iowa 52242, USA.

## Methods

Two studies, using female WKY and SH rats of 15–18 weeks of age, were done in a room with a 12 h light (0800–2000 h)–12 h dark cycle and an ambient temperature of 21 ± 1°C. Food and water were freely available.

In the first study, the continuous infusion of either saline (0·9% w/v; n = 6 rats) or clonidine HCl (10 $\mu$g $kg^{-1}$ $h^{-1}$, in terms of the base; n = 6 rats) was maintained for 10 days via ALZET osmotic minipumps (Model 2002; nominal pumping rate 0·5 $\mu$L $h^{-1}$ ALZA corporation, Palo Alto, California, USA) which were implanted (at 2400 h) subcutaneously under light halothane anaesthesia (2% in 100% oxygen). On day 5 of infusion the rats were reanaesthetized with a mixture of amylobarbitone (30 mg $mL^{-1}$) and methohexitone (16·7 mg $mL^{-1}$) given as 1 mL $kg^{-1}$ i.p. A cannula was inserted into the right carotid artery for the subsequent measurement of mean arterial blood pressure (MAP) and heart rate (HR), and for the withdrawal of blood (0·3 mL replaced with saline), and immediately on days 7, 9 and 10 of infusion between 1500–1800 h). At the end of the 10 day infusion (at 2400 h), the minipumps and blood were removed (0·3 mL replaced with saline) after 8–10, 12–15, 15–18 and 32–36 h, and changes in serum glucose and serum insulin determined. The resting MAP values of the control (saline-infused) WKY and SH rats were 120 ± 1 and 176 ± 2 mm Hg, respectively. The MAPs of the clonidine-infused WKY and SH rats were 110 ± 1 and 141 ± 4 mm Hg, respectively (−10 and −35 mm Hg different from respective controls, $P < 0·05$ for both comparisons).

In the second study, the rats (kept 3 to a cage) were divided into four groups (n = 6 rats per group). Two groups received clonidine (10 $\mu$g $kg^{-1}$ $h^{-1}$) and two groups received saline for 10 days via the subcutaneously implanted minipumps. At the end of the 10 day infusion (at 2400 h) rats from one saline- and one clonidine-infused group were decapitated and the trunk blood collected. In the other two groups, the minipumps were removed (under halothane) and the animals were decapitated 15–18 h later (i.e. between 1500–1800 h), the time representing the period of peak post-infusion withdrawal behaviour (Jarrott et al 1987). All the infusion and post-infusion groups were killed between 1500–1800 h on the same day. The body weights and temperatures were measured immediately before decapitation. The trunk blood or the samples collected via the arterial cannulae were allowed to clot at 4°C and then immediately centrifuged (3000 g for 5 min at 4°C). The resulting serum was then removed and stored at 0°C. The concentrations of insulin (ng $mL^{-1}$) were determined by radioimmunoassay (double-antibody) using rat insulin standards as described by Cameron et al (1973). The concentrations of glucose (mmol $L^{-1}$) were determined within 24 h by a YSI Glucose Analyzer (Model 23AM, Yellow Springs Instrument Company, Ohio, USA). Body temperatures were measured by means of a thermistor probe inserted 6 cm into the rectum and recorded with the aid of a telethermometer (Model 423, Yellow Springs Instrument Co, USA). All values represent the mean ± s.e.m. of the group data (n = 6 rats per group). The significance of the differences between means were determined by analysis of variance followed by

Student's modified $t$-tests with the Bonferroni adjustment for multiple comparisons (Wallenstein et al 1980).

## Results

The serum glucose concentrations of WKY and SH rats during days 7, 9 and 10 of infusion of either saline or clonidine and at selected times after the cessation of these infusions (study 1) are shown in Table 1. The concentrations of the control SH rats (i.e. those receiving saline) were significantly higher than those of the control WKY rats at every time. The concentrations of both the WKY and SH rats were significantly elevated on each day of clonidine infusion, the increases being similar on each of the three days ($P > 0{\cdot}05$ for between-day comparisons).

Following withdrawal of the clonidine infusions, the serum concentrations of glucose fell to below those of the controls within 8–10 h. Those of the clonidine-withdrawn groups of WKY and SH rats were significantly below their respective control values at 8–10 h, 12–15 h and 15–18 h post-infusion. In the clonidine-withdrawn WKY rats, these concentrations were equivalent to those of controls by 32–36 h (SH rats not measured).

There were no differences between the WKY and SH rats in terms of the increase in serum glucose concentrations during clonidine infusion or the reduction in these concentrations following the withdrawal of the infusion.

In the second study, also, the serum glucose concentrations of the control SH rats were significantly higher (+9%) than those of the WKY controls although there were no differences in insulin concentrations between the strains (Table 2). In addition, the SH controls had significantly higher body temperatures (+0·5°C) than those of the WKY controls but there were no differences in body weight. On day 10 of the continuous infusion of clonidine (10 $\mu$g kg$^{-1}$ h$^{-1}$), the serum glucose concentrations were elevated in the WKY (+26% of control) and SH (+18%) rats whereas the insulin levels were decreased in the WKY (−64%) and SH (−47%) rats. The WKY and SH rats receiving the clonidine infusion displayed a relative hyperthermia (+0·5°C and +0·5°C, respectively) which is due to a modulation of the circadian body temperature cycle (Maccarrone et al 1984). The body weights of these rats were not different to their respective controls.

Fifteen to 18 h after the cessation of the clonidine infusion, the serum glucose levels of the WKY and SH rats were lower than those of their respective controls (−18 and −24%, respectively) whereas the insulin levels remained suppressed in both strains (−52 and −58%, respectively). In addition, the post-clonidine infusion groups of WKY and SH rats displayed hyperthermia (+1·2°C and +1·1°C, respectively) and losses in body weight (−33 g and −24 g, respectively).

## Discussion

The present study demonstrates that the serum glucose levels of WKY and SH rats were elevated whereas the concentrations of insulin were decreased on day 10 of a continuous subcutaneous infusion of clonidine (10 $\mu$g kg$^{-1}$ h$^{-1}$). These findings are consistent with previous studies which have shown that the acute systemic injection of clonidine increased glucose concentrations via an increase in hepatic glycogenolysis (Rehbinder & Deckers 1968) and suppression of insulin secretion (Senft et al 1968; Ismail et al 1983), and perhaps a centrally-mediated increase in glucagon secretion (Zacny & Bugajski 1983). Moreover, the results also demonstrate that the effects of clonidine on the levels

Table 1. The serum glucose concentrations of WKY and SH rats during the infusion of either saline (0·9% NaCl) or clonidine (10 $\mu$g kg$^{-1}$ h$^{-1}$) and at selected times after the cessation of these infusions. Each value represents the mean ± s.e.m. (n = 6 rats per group).

| | | Day of infusion | | | Hours post-infusion | | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Treatment | 7 | 9 | 10 | 8–10 | 12–15 | 15–18 | 32–36 |
| WKY | saline | 6·6±0·2 | 7·0±0·1 | 6·8±0·2 | 7·3±0·1 | 6·9±0·2 | 7·2±0·1 | 6·8±0·2 |
| | clonidine | 8·0±0·2* | 8·7±0·2* | 8·3±0·3* | 6·5±0·2* | 5·9±0·1* | 5·8±0·2* | 6·5±0·2 |
| | % saline | +20% | +24% | +22% | −11% | −14% | −19% | +4% |
| SH | saline | 7·6±0·2† | 7·9±0·1† | 7·5±0·1† | 8·2±0·2† | 7·8±0·2† | 8·0±0·2 | ND |
| | clonidine | 8·7±0·2* | 9·2±0·3* | 8·5±0·2* | 6·9±0·3* | 6·6±0·2* | 6·1±0·3* | ND |
| | % saline | +14% | +16% | +13% | −16% | −15% | −24% | |

* $P < 0{\cdot}05$, clonidine & infusion or post-infusion versus saline controls.
† $P < 0{\cdot}05$, SH saline-infusion or post-infusion versus relevant WKY saline-infusion or post-infusion.
ND, not determined.

Table 2. The serum glucose concentrations, insulin concentrations, body temperatures (°C) and the body weights of WKY and SH rats on day 10 of clonidine infusion (10 $\mu$g kg$^{-1}$ h$^{-1}$) and 15–18 h after cessation of infusion. Each value represents the mean ± s.e.m. (n = 6 rats per group).

| | WKY | | | SH | | |
|---|---|---|---|---|---|---|
| Parameter | Control | Infusion | Post-Infusion | Control | Infusion | Post-Infusion |
| Glucose (mmol L$^{-1}$) | 6·9±0·1 | 8·7±0·2* | 5·7±0·2* | 7·5±0·2† | 8·9±0·2* | 5·8±0·1* |
| Insulin (ng mL) | 3·2±0·5 | 1·1±0·1* | 1·2±0·2* | 3·6±0·8 | 1·9±0·2* | 1·5±0·3* |
| Body temperature (°C) | 37·2±0·1 | 37·7±0·1* | 38·4±0·2* | 37·7±0·1† | 38·2±0·1* | 38·8±0·2* |
| Body weights (g) | 225±8 | 218±9 | 192±5* | 212±6 | 210±6 | 188±3* |

All groups were killed between 1500–1800 h on the same day (see Methods). The WKY and SH control groups each consisted of two subgroups. One subgroup consisted of 3 rats in which the saline containing minipumps were present at the time of decapitation. The other subgroup consisted of 4 rats in which the minipumps were removed 15–18 h earlier (see Methods). As all animals were killed on the same day (between 1500-1800 h) and no differences between the two subgroups were found ($P > 0{\cdot}75$ analysis of variance) the data were combined. *$P < 0{\cdot}05$, infusion or post-infusion versus control, † $P < 0{\cdot}05$, SH control versus WKY control.

of glucose were similar on days 7 through 10 of the continuous infusion of the drug suggesting tolerance does not occur during this time. It is evident that the hyperglycaemic effects of the clonidine infusion in the SH strain are qualitatively and quantitatively similar to those in the WKY animals, suggesting no dysfunction of $\alpha$-adrenoceptors systems regulating glucose metabolism in this strain.

The present results differ from those in normotensive and hypertensive humans. Although the acute administration of clonidine elevates blood glucose levels in normotensive humans, no disturbances in blood glucose or insulin levels or insulin release in response to ingested glucose are observed during chronic clonidine treatment in either normotensive or hypertensive subjects (see Boyar et al 1980; Stornello et al 1986). This suggests that, unlike the rat, the actions of clonidine on glucose metabolism in humans are subject to the development of tolerance.

The cessation of the clonidine infusion resulted in hypoglycaemia in both strains, even though their insulin levels remained suppressed, this suggesting post-infusion hypoglycaemia may result from mechanisms other than the actions of insulin. While its sustained suppression may result from one or a combination of factors, it is unlikely to be due to clonidine because this drug has a half-life of only ca 30 min in rats (Conway & Jarrott 1982). It is probable that the suppression may result from the lowered levels of circulating glucose, a suggestion supported by the study of Ishii et al (1985) who found that the rise in insulin levels in response to administered glucose was enhanced in rats withdrawn from clonidine. From their in-vivo and in-vitro studies, Ishii et al (1985) concluded that the enhanced release of insulin (in response to glucose) in the clonidine-withdrawn rats was mainly due to hyper-responsiveness of the pancreatic B-cells. In addition, the cessation of clonidine infusion (21 $\mu g\ kg^{-1}\ h^{-1}$ for 12 days) results in elevated plasma noradrenaline levels in both normotensive and SH rats (see Thoolen et al 1981) and this may be involved in the suppression of circulating insulin, since noradrenaline exerts a potent inhibitory effect on the rate of glucose-induced insulin secretion from the islets of Langerhans (Morgan & Montagne 1985).

The present results differ from those of Ishii et al (1985) with respect to changes in serum insulin and glucose concentrations. These authors found that glucose levels were not changed on the 10th day of clonidine treatment although, as in the present study, they found they were reduced on withdrawal of treatment. Moreoever, they found that, whereas insulin levels were suppressed during treatment (as we have found) these reduced levels were not sustained following withdrawal of clonidine.

The differences in the results of Ishii et al (1985) and ours may be because those authors obtained their data from chloralose-anaesthetized animals, or that they used a treatment regime that could not be regarded as truly chronic.

In conclusion, the present study demonstrates that the clonidine infusion and post-infusion phases are associated with disturbances in serum glucose and insulin levels. As such, these metabolic effects should be considered when evaluating the overall pharmacological profile of clonidine in experimental animals.

The authors wish to thank Dr M. R. Fennessy and Dr A. J. M. Verberne for useful discussions.

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