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Altered thermoregulatory responses to clonidine in streptozotocin-diabetic rats

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1 The effects of streptozotocin (STZ) treatment on α_2-adrenoceptor regulation of body temperature were studied by monitoring the response of colonic temperature to administration of clonidine.

2 A dose-dependent fall in colonic temperature occurred in control rats given clonidine challenge (0.05-2.0 mg kg^{-1}, s.c.); this response was inhibited by prior administration of either yohimbine or idazoxan (2 mg kg^{-1}, s.c.) but not by the peripherally-acting α_2-adrenoceptor antagonist L-659,066 (10 mg kg^{-1}, s.c.).

3 In rats treated with STZ (65 mg kg^{-1}, i.v.) administration of clonidine elicited a dose-independent hyperthermia (circa 1°C.); this effect was unaltered by prior administration of yohimbine or idazoxan.

4 Naloxone (5 mg kg^{-1}, s.c.) elicited a small fall in temperature(< 1°C.) in both control and STZ-treated rats; naloxone pretreatment did not alter the temperature response to clonidine in either group.

5 Nicotinic acid (10 mg kg^{-1}, s.c.) caused a similar small elevation in temperature in both groups.

6 Administration of replacement insulin to STZ-treated rats maintained weight gain and low blood glucose while the thermoregulatory response to clonidine slowly reverted to normal.

7 These results show that altered central temperature control is an element of the generalised abnormality of α_2 receptor function induced by STZ.

Keywords: Temperature; hypothalamus; diabetes; streptozotocin; clonidine; α_2-adrenoceptors

Introduction

In recent years there has emerged a growing appreciation of the possible central nervous system (CNS) effects of diabetes mellitus (Mooradian, 1988; Ryan, 1988; McCall, 1992). The effects of insulin-dependent diabetes mellitus (IDDM) on brain function are subtle in nature and comprise both direct metabolic changes and indirect actions through altered central regulatory mechanisms. The findings in human disease are complicated by the variety of pathological and therapeutic factors in each case, and the rat streptozotocin (STZ) model has been employed to make controlled studies of the CNS abnormalities associated with insulin lack. A range of centrally-regulated activities are altered by STZ-diabetes in rats; feeding, paradoxical sleep, analgaesia, submissive and avoidance behaviour are increased, whereas sexual behaviour and aggression are diminished compared to control animals (Leedom & Meehan, 1989). Studies of experimental diabetes help to elucidate the underlying pathophysiology and may also contribute to understanding normal physiological mechanisms.

There is a variety of evidence that diabetic animals have altered sensitivities to centrally-acting drugs. STZ-diabetic rats are less sensitive than normal to opioid analgesics (Simon & Dewey, 1981) or the behavioural effects of amphetamine and apomorphine (Rowland *et al.*, 1985). McKenzie & Trulson (1978) reported a blunted sensitivity to indirect 5-hydroxytryptamine (5-HT) receptor agonists, while an apparent subsensitivity to central diuretic and natriuretic effects of the α_2-adrenoceptor agonist clonidine has also been reported in STZ-treated rats (Zhang & Patel, 1991).

Chu *et al.* (1986) found diabetic animals to have impaired ability to adapt to altered ambient temperature. While thermoregulation might be defective at the periphal or central level in diabetes, the fact that the hypothalamus is the single region of the CNS likely to be directly sensitive to lack of peripheral insulin (Leedom & Meechan, 1989), and the involvement of the hypothalamus in several other functions altered in diabetes, suggests that an effect at an hypothalamic site could underlie the abnormality in temperature regulation. The sensivity of α_2-adrenoceptors may be pharmacologically monitored by challenge with acute clonidine, followed by measurement of temperature response (Zacny, 1982; Livingstone *et al.*, 1984; Colpaert, 1986). The present investigation examined the effects of STZ diabetes on the temperature response to clonidine in rats. The nature of the clonidine effect was studied by use of selected receptor anatagonists, and also the effects of insulin replacement following STZ treatment.

Methods

Animals

All experiments employed adult male Wistar rats of initial weight 200-250 g. Animals were fed standard laboratory diet and water *ad lib* and were housed at ambient temperature 22-24°C. Administration of STZ was performed under ether anaesthesia. Freshly-prepared STZ solution was injected at a dosage of 65 mg kg^{-1} via the tail vein.

Insulin treatment

Protamine zinc insulin (Ultralente, Novo) was administered s.c.. The dosage was initially 4 units daily at 16 h 00 min and the daily dose was subsequently adjusted in the range 3-6 units depending on the blood glucose control being achieved.

Blood glucose

Blood for glucose determination was obtained from the tail, 0.1 ml of freshly-drawn blood was added to 1 ml of TCA (5%)

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and thoroughly mixed. Samples were centrifuged (3,000 g min) and an aliquot of supernatant assayed for glucose by the glucose oxidase method (Reanal assay kit). Results were expressed as mmol l^{-1} whole blood.

Body temperature

Temperature was measured as colonic temperature. Animals were gently restrained in a polypropylene holder to which they had been previously acclimatised. A digital thermistor thermometer (Omron, model MC-3B, accuracy ± 0.1°C.) was inserted 2.5 cm rectally and temperature recorded at equilibrium. Animals were returned to the home cage after measurement. For comparison of basal temperature in control and STZ-treated animals, temperatures were recorded at hourly-intervals over a 24 h period.

Clonidine challenge

An initial temperature reading was taken ($t=0$ min) and drug or saline vehicle injected immediately. Further temperature measurements were made at $t=30$, 60, 90 and 120 min post-injection. Where an antagonist drug was being tested in combination with clonidine, the antagonist was administered s.c. 15 min prior to the clonidine challenge. Yohimbine or idazoxan were used at 2 mg kg^{-1}, naloxone at 5 mg kg^{-1} and L-659,066 at 10 mg kg^{-1}. Nicotinic acid was administered at 10 mg kg^{-1} s.c. and temperature recorded at 15 and 30 min post-injection.

Drugs

Clonidine, yohimbine and idazoxan were purchased from Sigma, and nicotinic acid from Gideon Richter. The peripheral α_2 antagonist, L-659,066 (a spirocyclic-substituted benzofuroquinolizine) was a gift of Merck, Sharpe & Dohme, West Point.

Statistical procedures

All experiments employed age-matched control and treated groups of 6–8 rats. Basal temperatures were calculated as group means. The significance of any temperature change within a group was assessed by paired-sample t test. For between-group comparison of temperature responses to challenge with clonidine, temperature changes ($\triangle$T) from initial value at each interval following injection were calculated as means ± s.e. of mean for each group. The significance of any differences between control and treated groups was assessed by Student's unpaired t test (2-tailed).

Results

Body weight and blood glucose effects of STZ and insulin

Table 1 shows the repsonse of blood glucose concentration to administration of STZ with or without subsequent insulin. At 5 days post treatment the blood glucose concentration was 3–4 times the pretreatment value and over the 7 weeks period of weekly monitoring the animals showed effectively stable blood glucose status, while failing to gain weight and developing the expected polyuria, polydipsia and other gross signs of IDDM. Animals immediately started on daily insulin administration continued to gain weight and showed no signs of insulin depletion, and mean blood glucose concentration did not rise. While the insulin-repleted animals showed all characteristics of normal insulin status, some animals died over the period of this study, reflecting the difficulty of adequately replacing pancreatic hormone, while the animals without insulin all stabilized at a hyperglycaemic state. Blood glucose concentration was markedly more uniform in the diabetic group than in the insulin-repleted animals.

Basal temperature in STZ-treated rats

At 12 days following STZ administration, basal temperatures were measured in treated rats and a matched group of control animals housed under identical conditions. Measurements were made over a 24 h period and the results are illustrated in Figure 1. The time-course of mean temperature was similar in both groups, showing raised temperatures during the period 20 h00 min-04 h00 min with a fall towards 08 h00 min, at which time temperatures again rose in the controls, to fall towards 12 h00 min and remain relatively constant until 20 h00 min. Only at 09 h00 min-11 h00 min were the tem-

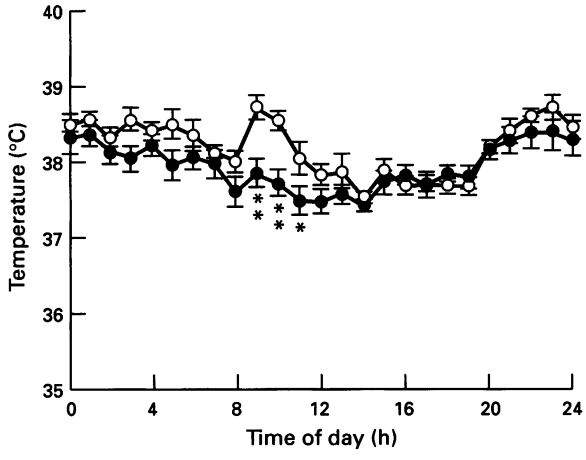

Figure 1 Time-course of variation in colonic temperature of control (○) and diabetic (●) rats over a 24 h period. Diabetic rats were 12 days post-streptozotocin administration. Temperatures are mean ± s.e. mean of six animals $^{**}P<0.01$; $^{*}P<0.05$, diabetic significantly different from control

Table 1 Weight and blood glucose concentration (mean ± s.e.mean) in streptozotocin-treated rats with (+) or without (–) insulin replacement

Days	*Body weight* (g) +	*Body weight* (g) –	*Glucose* (mmol l^{-1} whole blood) +	*Glucose* (mmol l^{-1} whole blood) –
post-treatment				
5	214 ± 3.9 (20)	193 ± 4.7 (10)	3.0 ± 0.31	19.9 ± 1.14
12	256 ± 5.3 (18)	199 ± 4.7 (10)	4.4 ± 1.10	22.2 ± 1.68
21	280 ± 3.9 (15)	201 ± 4.4 (10)	3.2 ± 0.93	24.0 ± 1.27
28	293 ± 3.5 (12)	202 ± 3.9 (10)	3.5 ± 1.36	22.2 ± 1.08
35	316 ± 4.8 (10)	210 ± 4.7 (10)	4.2 ± 1.08	21.2 ± 0.89

Control (pretreatment) values were 192 ± 5.5 g and 5.6 ± 0.68 mmol l^{-1} (30).

peratures in the STZ group significantly below normal. Experimental challenges were performed within the period 10 h00 min-15 h00 min, the small difference in basal temperature between control and diabetic animals being negligible in comparison to the responses elicited by clonidine.

Thermoregulatory responses to clonidine

Control animals' temperature responses to clonidine injection are summarised for a range of doses in Figure 2a. Compared to saline vehicle, clonidine elicited a dose-dependent hypothermia, with significant lowering of temperature ($\triangle$T) sustained throughout the monitoring period ($P<0.05$ or greater for all doses and times post-injection). Increasing dose was also associated with signs of sedation and piloerection. The temperature responses to the same range of clonidine doses in STZ-diabetic rats (15 days or more post-STZ) are shown in Figure 2b. While the lowest dose of clonidine did not significantly affect temperature, significant hyperthermia ($P<0.05$ or greater at all time post-injection) occurred in response to doses of 0.1 mg kg^{-1} or greater. Notably, this elevation in temperature was modest and not dose-related, while being sustained throughout the test period in a similar way to control responses.

Effects of blocking drugs

Control and STZ-diabetic rats were pretreated with one of the blockers yohimbine, idazoxan, or naloxone and then challenged with clonidine(0.1 mg kg^{-1}); control animals were also pretreated with L-659,066 The effects of pretreatment on the clonidine response of control rats are shown in Table 2. The 90 min temperature falls were significantly altered by prior idazoxan or yohimbine, but not affected by the peripheral blocker L-659,066 or by naloxone. When administered alone at these concentrations, idazoxan, yohimbine and L-659,066 did not evoke significant temperature changes in control animals, while naloxone alone caused a small fall in temperature which did not differ in control and diabetic animals (mean and s.e. -0.78 ± 0.17 and $-0.80\pm0.28°$, respectively). The hyperthermia ($\triangle$T at 90 min) in diabetic rats was not significantly altered by pretreatment with idazoxan or yohimbine (mean and s.e. 0.48 ± 0.12 and 0.78 ± 0.18 respectively vs. a control value $0.52\pm0.21°$).

Time-course of STZ effect on clonidine response

The development of the altered thermoregulatory response to clonidine challenge (0.1 mg kg^{-1}) was followed in the days immediately post STZ administration, and the results are shown in Table 3. The magnitude of the clonidine hypothermia was significantly reduced from the pre-STZ values at two days post-injection, and the response had further altered to become a hyperthermia by 12 days. Blood glucose concentrations in these animals were 18.41 ± 4.1 and 24.2 ± 2.2 mmol l^{-1} (mean and s.e. mean $n=6$) at 4 and 12 days post-STZ, respectively).

Effects of insulin replacement

The effects of insulin replacement in STZ-treated rats were also assessed by challenge with 0.1 mg kg^{-1} clonidine, and the re-

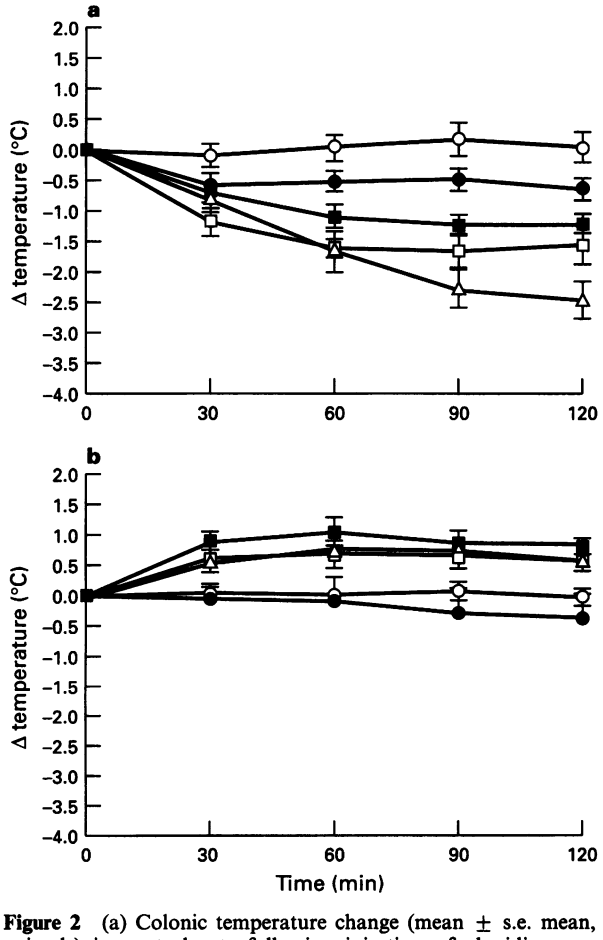

Figure 2 (a) Colonic temperature change (mean $\pm$ s.e. mean, 6–8 animals) in control rats following injection of clonidine s.c.: (○) saline control; (●) 0.05; (■) 0.1; (□) 0.5; (△) 2mg kg^{-1}. (b) Colonic temperature change in diabetic rats following injection of clonidine s.c. Symbols as for (a). Rats were challenged at 15 or more days post-streptozotocin administration

Table 2 Effects of pretreatment with yohimbine, idazoxan, L-659,066 or naloxone on the thermoregulatory response to clonidine challenge (0.1 mg kg^{-1} s.c.) in control rats

Pretreatment (t-15 min)	*ΔT (t+90 min)* (°C, mean $\pm$ s.e.mean, $n=8$)
Saline vehicle	-2.11 ± 0.23
Idazoxan (2 mg kg^{-1}, s.c.)	$+0.630.15$*
Yohimbine (2 mg kg^{-1}, s.c.)	-0.55 ± 0.21*
L-659,066 (10 mg kg^{-1}, s.c.)	-1.73 ± 0.47
Naloxone (5 mg kg^{-1}, s.c.)	-2.18 ± 0.42

Administration of yohimbine, idazoxan or L-659,066 alone caused no significant change in temperature compared to saline vehicle. Naloxone alone led to a small fall in temperature which did not differ in diabetic and control rats (-0.78 ± 0.17 and -0.80 ± 0.28, respectively).
*Significantly different from saline control, $P<0.01$.

Table 3 Time-course of development of altered thermoregulatory response to clonidine (0.1 mg kg^{-1} s.c.) following treatment with streptozotocin

	ΔT (t+90 min) (°C, mean $\pm$ s.e.mean, $n=8$)
Control (pre-STZ)	-1.50 ± 0.50
Post-STZ day 1	-1.56 ± 0.22
2	-0.65 ± 0.16*
3	-0.83 ± 0.24*
12	$+1.00\pm0.18$*

*Significantly different from control, $P<0.01$

sults are summarised in Table 4. The response had altered significantly from control by two days, in a similar manner to the change occurring without insulin. After 10 days the response had altered further from control, but at 15 days and at 33 days of insulin replacement the response to clonidine tended to return to the hypothermia of control rats, although the sensitivity was not fully restored to the pre-STZ state. Blood glucose concentrations in the insulin-treated rats were 8.78 ± 0.73, 2.43 ± 0.41 and 3.07 ± 0.70 mmol l^{-1} at 4, 10 and 15 days, respectively, post STZ (mean and s.e. mean $n = 6-8$).

Responses to nicotinic acid

Nicotinic acid was administered s.c. at 10 mg kg^{-1} and temperature monitored at intervals of 15 and 30 min post-injection, since any temperature responses to the peripheral vasodilator action were expected to be rapidly manifested. Nicotinic acid caused small increases in temperature at 15 and 30 min which were very similar in both groups (0.73 ± 0.25 and 0.83 ± 0.31 in controls: 0.53 ± 0.06 and 0.62 ± 0.12 in STZ rats; challenge performed 35 days post-STZ).

Discussion

The results of these experiments clearly demonstrated that in STZ-induced IDDM the thermoregulatory reponses of rats were altered as shown both by basal temperature and sensitivity to the α_2-adrenoceptor agonist, clonidine. The effect of STZ on basal temperature was subtle and limited to effects detected at an inversion point in the normal daily pattern. The dark-light transition is accompanied by altered activity and secretion (e.g. of corticosterone) and the basal temperature difference plausibly represents an expression of the impaired adaptive capability of diabetic animals shown by several environmental response studies (Chu *et al.*, 1986; Bellush & Henley, 1990; Kilgour & Williams, 1994).

Although clonidine administered peripherally could influence temperature by peripheral or central actions, the effect is thought to be primarily central (Buccafusco, 1992). Administration of noradrenaline or clonidine to the preoptic area of the guinea-pig hypothalamus elicits hypothermia that is blocked by coadministration of yohimbine or rauwolscine (Quan *et al.*, 1992). Earlier studies have shown that the hypothermia caused by low dose clonidine (0.05 mg kg^{-1}, i.p.) was antagonized by central or periphal yohimbine (Zacny, 1982). The selective α_2-agonists, UK-14,304 and BHT 933, also elicit hypothermia by central actions (Colpaert, 1986; Bill *et al.*, 1989). In the present studies, the hypothermia induced in control rats by clonidine was inhibited by the centrally-acting blockers yohimbine or idazoxan but not by L-659,066 which does not readily penetrate CNS (Clineschmidt *et al.*, 1988). The lack of inhibitory effect of naloxone indicated that the hypothermia elicited by clonidine does not depend on opioid-mediated mechanisms.

The lack of sensitivity to an α_2-induced hypothermia in STZ-treated rats could also be due to abnormality at central or peripheral sites, since the hypothalamic control is exercised through reduction in metabolic heat production (Quan *et al.*, 1992). The similarity of the response to the peripheral vasodilator nicotinic acid would appear to exclude gross vascular changes in diabetes as causes of the inversion of the thermoregulatory response to clonidine. An altered sensitivity of hypothalamic α_2 receptors to clonidine could contribute at least in part to the changes found following STZ, and such a blunting would be consistent with other findings on the effects of the toxin on central responsiveness to clonidine (Locatelli *et al.*, 1986; Zhang & Patel, 1991) or dexmedetomidine (Guo *et al.*, 1991). While there is evidence that STZ-diabetes alters peripheral α_2-linked functions in rat liver (Allard *et al.*, 1991) and intestine (Ramabadran *et al.*, 1990), there is also much evidence that this condition causes reduced release and turnover of hypothalamic noradrenaline (Oliver *et al.*, 1989; Lackovic *et al.*, 1990; Shimizu, 1991).

The inversion of the temperature response to a hyperthermia may also be explicable on the basis of noradrenergic abnormalities at hypothalamic sites. In non-diabetic guinea-pigs damage to the pre-optic area leads to hyperthermia and clonidine microinjection leads to a biphasic temperature response, the hyperthermia being dose-dependent (Romanovsky *et al.*, 1993). Animals depeted of central noradrenaline by reserpine treatment also show a thermogenic response to clonidine, by an apparently postsynaptic α_2-mechanism (Bill *et al.*, 1989). Thus STZ-treated rats may be considered to manifest a milder form of the hypothalamic noradrenergic deficit and basal hypothermia seen in reserpinised animals. If, however, the hyperthermia in response to clonidine were mediated by postsynaptic α_2-receptors, then this effect should have been blocked by α_2-antagonists, which was not the case. The hypothalamic defects associated with STZ-treatment may also arise at post-receptor levels, as suggested for the locus coeruleus (Guo *et al.*, 1991). Irrespective of the specific cellular site of the STZ effect, these results directly show that STZ diabetes leads to disruption of the normal responsiveness to α_2-adrenoceptor activation of a thermoregulatory process, and offer an explanation of the purely behavioural findings on the impaired ability of diabetic animals to withstand environmental challenges. Alteration of α_2-mediated mechanisms thus accounts for several separate STZ effects, and is an indication that these functions share a common STZ-sensitive element in normal physiological regulation.

The alteration in response to clonidine developed in the days following STZ treatment in a similar manner in rats with or without insulin replacement, indicating that hyperglycaemia was not an essential causative factor. Insulin-repleted rats only reverted to normal α_2-agonist responsiveness between 15 and 33 days of daily insulin therapy, despite showing normal or low blood glucose and normal weight gain. The defect in thermoregulation in STZ-diabetes appears to be a slowly reversible hypothalamic or associated lesion independent of carbohydrate metabolic regulation.

Table 4 Influence of insulin replacement (3–6 i.v. daily) on thermoregulatory response to clonidine (0.1 mg kg^{-1} s.c.) in rats treated with streptozotocin

	ΔT ($t+90$ min) (°C, mean ± s.e.mean, $n=8$)
Control (pre-STZ)	-1.24 ± 0.25
Days + insulin 2	-0.54 ± 0.28*
10	$+0.01 \pm 0.15$*
15	-0.16 ± 17*
33	-0.88 ± 0.14

*Significantly different from pre-STZ control: , $P<0.01$

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