Effects of chronic bromocriptine (CB-154) treatment on the plasma glucose and insulin secretion response to neurocytoglucopenia in rats

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Abstract

Neurocytoglucopenia has been reported to increase both parasympathetic and sympathetic tone with a predominant effect on the latter, which accounts for the major effect of plasma hyperglycemia and the inhibition of insulin secretion. The aim of this study was to determine the effects of chronic treatment with bromocriptine (0.4 mg/100 g body wt per day), a potent sympatholytic D₂-dopaminergic agonist, on hyperglycemia and insulin secretion in response to neurocytoglucopenia induced by 2-deoxy-D-glucose (2DG). After 2 weeks of bromocriptine treatment the animals, freely moving in their cages, were submitted to 2DG administration (50 mg/100 g body wt) via atrial catheter infusion. After 2DG infusion, the plasma prolactin of vehicle-treated (VEH) rats increased rapidly, reaching a peak at 10 min (34.3 ± 7.6 ng/ml; $P < 0.01$). In contrast, 2DG infusion failed to induce any significant change in the plasma prolactin levels of bromocriptine-treated (BR) rats. BR rats showed higher

resting glucose levels than control rats (8.2 ± 0.28 mM (BR) vs 6.0 ± 0.18 mM (VEH); $P < 0.01$). However, the hyperglycemic response of BR rats to 2DG injection was 30% lower than that of VEH rats ($P < 0.05$). BR rats also showed a rapid rise in plasma insulin levels reaching a peak at 30 min after 2DG injection (243% higher than basal values; $P < 0.01$). This increased rise in the insulin response to neurocytoglucopenia of BR rats was blocked by previous intravenous injection of atropine methyl nitrate (0.2 mg/100 g body wt).

The present results suggest that chronic treatment with bromocriptine determines a strong increase in the parasympathetic tone response to neurocytoglucopenia, which is responsible for the higher stimulation of insulin secretion observed in BR rats. The data also provide further evidence that D₂-dopaminergic agonist can block neurocytoglucopenia-induced prolactin release.

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Introduction

Neurocytoglucopenia activates hypothalamic glucoreceptors, with a consequent increase in sympathetic outflow to liver, pancreas, adrenal medulla and adipose tissue, resulting in increased hepatic glucose production, insulin inhibition and free fatty acid mobilization from adipose tissue (Coimbra *et al.* 1979, Coimbra & Migliorini 1983, 1986). In support of this concept was the finding that, in the rat, neurocytoglucopenia induced by administration of 2-deoxy-D-glucose (2DG) results in a significant positive correlation between hypothalamic noradrenergic neuronal activity and blood glucose concentration (Smythe *et al.* 1984, Storlien *et al.* 1985). 2DG is a non-metabolizable glucose analogue which effectively blocks the utilization of glucose in neurons (Brown 1962, Havel & Taborsky 1989). Neurocytoglucopenia induced by 2DG also

increases prolactin secretion which can be blocked by apomorphine, a dopaminergic agonist (Okajima *et al.* 1980). Neurocytoglucopenia has been reported to increase both parasympathetic and sympathetic tone, with a predominant effect on the latter, which accounts for the major stimulation of hepatic glucose production and the inhibition of insulin secretion. In addition, it has been shown that when 2DG is infused into the hypothalamus (Peinado & Myers 1987) the efflux of either noradrenaline or dopamine is enhanced in areas that influence plasma glucose and insulin secretion.

These results support the hypothesis of a dopaminergic influence on the control of glucose and insulin secretion response to neurocytoglucopenia and to other stress situations. However, little attention has been paid to the participation of dopaminergic tone in this control despite the wide clinical use of dopaminergic agents, such as

bromocriptine, to inhibit prolactin secretion. Bromocriptine also activates hepatic gluconeogenesis in rats (Schmidt *et al.* 1983), reduces body fat stores and improves glucose tolerance in obese subjects (Cincotta *et al.* 1991, Cincotta & Meier 1996). Furthermore, bromocriptine is a potent sympatholytic D₂-dopaminergic agonist and the clinically most utilized therapeutic agent that inhibits prolactin secretion in males and females of all mammalian species tested so far. On the other hand, prolactin has been shown to increase β -cell proliferation, insulin synthesis and glucose-stimulated insulin secretion in rats (Reis *et al.* 1997, Sorenson & Brelje 1997). The glucose and insulin response to neurocytoglucopenia of animals chronically treated with a dopaminergic agonist is therefore of great physiological interest.

The experiments described in the present report were designed to determine the effect of the increased dopaminergic tone brought about by chronic treatment with bromocriptine (CB-154) on the hyperglycemic and insulin secretion response to the neuroglucopenic stress induced by 2DG. However, bromocriptine-treated rats showed higher resting glucose levels than control rats in addition to an abrupt and marked rise in plasma insulin elicited by the hyperglycemia induced by 2DG injection. Thus, the present study was also designed to explore the role of the cholinergic tone on the pancreas in this process. Since methylatropine is not effective in blocking central muscarinic receptors (Brezenoff *et al.* 1988), and the hyperglycemic and insulin response to 2DG during the light period when injected peripherally (Yamamoto *et al.* 1988), it was used to block the cholinergic brain-peripheral links.

Materials and Methods

Animals

Adult male Wistar rats (12 weeks) were used in these experiments. They had free access to Purina rat chow and water and were housed in temperature-controlled quarters with 14 h of light (5–19 h) per day. At the age of 9 weeks they were treated with CB-154 (BR; Sandoz, Basel, Switzerland), a dopamine agonist, or its diluent. BR was dissolved in sterilized water and injected i.p. daily at a dose of 0.4 mg/100 g body wt for 2 weeks. Atrial catheters were then inserted through the jugular vein under ether anesthesia, as described by Harms & Ojeda (1974). The venous cannulation was performed 1 or 2 days before the experiments and the catheter was kept patent by instillation of 1 ml heparinized saline (25 U/ml; Lique mine, Roche, Rio de Janeiro, Brazil).

Intravenous 2DG administration

On the day of the experiments the rats had their venous catheter rinsed and connected to a polyethylene tube (PE

50) filled with heparinized saline (10 U/ml). The animals were then returned to their home cages 1 h prior to i.v. infusion of 2DG (Sigma, St Louis, MO, USA) or saline control. The rats were freely moving and were not handled from this time until the end of the tests. They were deprived of food and water throughout the experiments, and blood samples (0.3 ml) were collected at –2, 5, 10, 15 and 30 min while the animals were freely moving in the cage. At time-zero, 2DG (50 mg/100 g body wt in 10% solution) was infused over a period of 60 s. Saline solution (0.15 M NaCl) was used as control.

Peripheral cholinergic blockade

In these experiments, the venous catheters of the rats were rinsed regularly and the rats were allowed to rest for 1 h as usual. A blood sample was then collected and atropine methylnitrate (ATR) was injected (0.2 mg/100 g body wt i.v.). A 20-min postinjection period was allowed to elapse, after which 2DG i.v. was infused using the same procedure as described above.

Assays and statistics

Glucose was assayed in duplicate by the glucose oxidase method (GOD-ANA; Lab Test, Lagoa Santa, Brazil). Insulin was measured by RIA (kindly supplied by Luiz Flavio Leite of Novo Nordisk, Bagvaerd, Denmark) using rat insulin as standard, human ¹²⁵I-labeled insulin as a tracer and ethanol separation of the bound and free fractions. The average intra- and interassay coefficients of variation were 3.0% and 11.4% respectively. Plasma prolactin was measured in duplicate by RIA using materials kindly supplied by the NIDDK (Bethesda, MD, USA). The samples were run in the same assay with a sensitivity of 2 ng/ml plasma and the intra-assay coefficient of variation of 8%.

Differences between groups (area under the curves) were checked by ANOVA followed by the Newman-Keuls test. Values from samples taken after 2DG injection were compared with basal values using a repeated measure ANOVA and checked by paired *t*-test.

Results

Effects of neurocytoglucopenic stress on prolactin levels

Plasma prolactin levels were measured in order to evaluate the intensity of BR inhibition on prolactin secretion. As illustrated in Fig. 1, the basal prolactin levels of the groups treated with BR and 2DG (BR/2DG) and BR and saline (BR/SAL) were not significantly different from the control group treated with vehicle (VEH) and 2DG (VEH/2DG). After the infusion of 2DG, prolactin levels increased rapidly in the VEH/2DG group reaching the

Figure 1 Effect of i.v. administration of 2DG (50 mg/100 g body wt) or SAL on plasma prolactin levels of rats chronically treated with bromocriptine (BR/2DG, 10 rats and BR/SAL, 6 rats) or with vehicle (VEH/2DG, 9 rats). Data are plotted as means $\pm$ S.E.M. * $P < 0.05$ vs basal values; ** $P < 0.01$ vs basal values; + $P < 0.01$ vs the BR/2DG or BR/SAL group.

highest values at 10 min (34.3 ± 7.6 ng/ml), when the increase was about 238% of the initial value ($P < 0.01$). On the other hand, 2DG failed to induce any significant change in plasma prolactin levels in the BR/2DG group. Similarly the plasma prolactin levels of the BR/SAL group did not change after saline infusion.

Effects of neurocytogluopenic stress on plasma glucose levels

The groups of rats treated with the dopaminergic agonist BR showed significantly higher resting plasma glucose levels than the VEH-treated group (7.8 ± 0.28 , BR/2DG; 8.6 ± 0.28 , BR/SAL vs 6.0 ± 0.16 mM, VEH/2DG; $P < 0.01$). However, after 2DG infusion there was a strong increase in blood glucose levels of both BR/2DG and VEH/2DG groups (Fig. 2A). At 5 min they were already significant ($P < 0.01$), but increased much more and reached a peak at 30 min (14.5 ± 0.39 mM, BR/2DG vs 16.7 ± 0.83 mM, VEH/2DG). Although both groups showed significant hyperglycemic responses to neurogluopenic stress the BR/2DG group showed lower hyperglycemic increments when compared with the VEH/2DG group. This effect can be more accurately observed by the analysis of Fig. 3A in which the 30-min integrated area under the glucose curve was much lower

Figure 2 Effect of i.v. administration of 2DG (50 mg/100 g body wt) or SAL on plasma glucose levels of rats chronically treated with BR (BR/2DG, 10 rats and BR/SAL, 6 rats), with VEH (VEH/2DG, 9 rats) or with BR and ATR (BR/ATR/2DG, 11 rats and BR/ATR/SAL, 6 rats). Data are reported as means $\pm$ S.E.M. Preinjection levels (mean $\pm$ S.E.M.) of plasma glucose were 8.6 ± 0.3 mM for group BR/SAL, 6.0 ± 0.2 mM for group VEH/2DG, 7.8 ± 0.3 mM for group BR/2DG, 7.7 ± 0.6 mM for group BR/ATR/SAL, and 7.3 ± 0.2 mM for group BR/ATR/2DG. * $P < 0.01$ vs basal values; + $P < 0.01$ vs BR/SAL or BR/ATR/SAL; $\Delta P < 0.01$ vs BR/2DG.

in the BR/2DG group than in the VEH/2DG group ($P < 0.01$). After infusion of 0.15 M NaCl there was no significant change in blood glucose levels.

The effect of peripheral cholinergic blockade caused by ATR injected i.v. on 2DG-induced changes in blood glucose in BR-treated rats (BR/ATR/2DG group) is

Figure 3 Integrated areas under the glucose (A) and insulin (B) curves (shown in Figs 2 and 4) of BR- (BR/SAL, BR/2DG, BR/ATR/SAL and BR/ATR/2DG groups) or VEH-treated rats (VEH/2DG) submitted to injection of 2DG or SAL. * $P < 0.01$ vs the BR/2DG group; + $P < 0.01$ vs the BR/SAL or BR/ATR/SAL group; $\Delta P < 0.01$ vs the VEH/2DG group; ● $P < 0.01$ vs the BR/ATR/2DG group.

Figure 4 Effect of i.v. administration of 2DG (50 mg/100 g body wt) or SAL on plasma insulin levels of rats chronically treated with BR (BR/2DG, 10 rats and BR/SAL, 6 rats), with VEH (VEH/2DG, 9 rats), or with BR and ATR (BR/ATR/2DG, 11 rats and BR/ATR/SAL, 6 rats). Data are reported as means $\pm$ S.E.M. Preinjection levels (mean $\pm$ S.E.M.) of plasma insulin were 17.5 ± 3.3 μ U/ml for group BR/SAL, 28.0 ± 3.7 μ U/ml for group VEH/2DG, 41.0 ± 9.4 μ U/ml for group BR/2DG, 21.0 ± 3.0 μ U/ml for group BR/ATR/SAL, and 24.0 ± 2.5 μ U/ml for group BR/ATR/2DG. * $P < 0.01$ vs basal values; + $P < 0.01$ vs BR/SAL or BR/ATR/SAL; $\Delta P < 0.01$ vs BR/2DG.

shown in Fig. 2B. After 2DG injection, the BR/ATR rats showed the same glucose increments as those observed in VEH-treated rats injected with 2DG. ATR pretreatment resulted in a significant increase in the hyperglycemic response to 2DG injection in BR-treated rats when compared with BR-treated rats without cholinergic blockade ($P < 0.01$; Figs 2B and 3A).

Effect of neurocytoglucopenic stress on plasma insulin levels

As illustrated in Fig. 4A, 5 min after 2DG infusion there was a significant decrease in insulin levels in the VEH/2DG group (34% lower than initial values, $P < 0.05$); this was followed by a slow increase in insulin levels throughout the experiment (82% higher than initial values, $P < 0.05$). The BR/2DG group did not show the stress-induced insulin decrease generally observed following 2DG infusion but, in contrast, showed a strong and rapid rise throughout the experiment. At 10 min this increase was already significant (33% higher than initial values, $P < 0.05$) and reached a very high peak at 30 min (243% higher than initial values, $P < 0.01$). As illustrated by the 30-min integrated area under the incremental insulin curve (Fig. 3B), total insulin secretion was much higher in the BR/2DG group than in the VEH/2DG group ($P < 0.01$). After 0.15 M NaCl there was no significant change in plasma insulin levels.

The effect of peripheral cholinergic blockade by ATR on insulin secretion in response to neurocytoglucopenia in BR-treated rats (BR/ATR/2DG group) is shown in Figs 3B and 4B. After 2DG injection the BR/ATR/2DG rats showed the same insulin secretion responses as those observed in VEH-treated rats injected with 2DG. ATR pretreatment blocked the hyperinsulinemic response to 2DG injection in the BR/ATR/2DG group when compared with BR-treated rats without cholinergic blockade (BR/2DG group).

Discussion

The experiments reported here demonstrate that chronic treatment with the potent D_2 -dopamine agonist bromocriptine determined a strong insulin secretion in response to the hyperglycemia induced by 2DG neurocytoglucopenia. In contrast, rats chronically treated with vehicle showed the expected inhibition of insulin secretion and a fast rise in plasma glucose levels. This hyperglycemic response to 2DG overcame the sympathetic inhibition of insulin secretion only 30 min after 2DG administration when glucose reached a peak 272% higher than the basal values. Our data suggest that, during neurocytoglucopenia, parasympathetic tone predominates over sympathetic tone in bromocriptine-treated rats upon regulation of insulin secretion. The increased parasympathetic tone in bromocriptine-treated rats was mainly indicated by the strong insulin secretion, which was completely blocked by methylatropine pretreatment. Methylatropine is a cholinergic muscarinic blocker that is not effective in blocking central muscarinic receptors when injected peripherally (Brezenoff *et al.* 1988).

Hyperglycemic response to 2DG has been reported to be neurogenic (Frohman *et al.* 1973, Havel & Taborsky 1989, Matsunaga *et al.* 1989, Smythe *et al.* 1989, Carlsson *et al.* 1992). It is also known that 2DG increases both parasympathetic and sympathetic tone (Frohman *et al.* 1973, Carlsson *et al.* 1992). The predominant effect is of the latter which accounts for the major effect of plasma hyperglycemia and the inhibition of insulin secretion (Frohman *et al.* 1973, Storlien *et al.* 1985, Havel & Taborsky 1989, Smythe *et al.* 1989). The inhibition of insulin secretion by 2DG occurs via the hypothalamus and is mediated by the release of adrenal catecholamines (Frohman *et al.* 1973, Smith *et al.* 1973, Storlien *et al.* 1985, Havel & Taborsky 1989, Smythe *et al.* 1989). There are many studies showing that stimulation of D_2 -dopaminergic receptors in the brain may have some influence on blood glucose regulation by a mechanism which is dependent upon catecholamine release from the adrenal medulla (Quik & Sourkes 1977, Saller & Kremer 1991).

It is important to point out that neuroglucopenia induced by 2DG injection directly affects monoamine

(noradrenaline and dopamine) turnover and release in the hypothalamic neurons of paraventricular nucleus, dorso-medial nucleus, lateral hypothalamus, and ventromedial nucleus (VMH) (McCaleb & Myers 1982, Peinado & Myers 1987, Smythe *et al.* 1989). All these hypothalamic areas have been shown to be involved in the regulation of blood glucose and insulin levels via modulation of sympathetic and parasympathetic output (DeJong *et al.* 1977, Campfield *et al.* 1986, Smith & Campfield 1986, Shimazu 1987, Ionescu *et al.* 1989, Smythe *et al.* 1989, Steffens *et al.* 1991). Furthermore, it has been shown that disturbances of noradrenaline and dopamine neurons in the hypothalamus are involved in hypothalamic obesity (Takahashi *et al.* 1994) and an increment in the dopamine system in the central nervous system may contribute to the irregular VMH autonomic control of insulin secretion. We should like to emphasize that among the alterations caused by hypothalamic obesity induced by VMH electrolytic lesions are an increased parasympathetic neural input and an increased insulin secretion in response to glucose (Campfield *et al.* 1986, Smith & Campfield 1986), whose intensity is similar to that observed in the present experiment. Our results suggest that bromocriptine treatment may interfere with the neural regulation of blood glucose and insulin secretion via the autonomic nervous system, particularly via direct parasympathetic innervation of the islet of Langerhans. In addition, bromocriptine activation of hepatic gluconeogenesis and consequent increase in resting plasma glucose levels (Schmidt *et al.* 1983) may have improved β -cell responsiveness to parasympathetic stimulation. It has been demonstrated that chronic hyperglycemia brings about changes in the activity of the autonomic nervous system which enhanced β -cell responsiveness to glucose *in vivo* (Zawalich *et al.* 1989, N'Guyen *et al.* 1994). These data agree with experiments showing that the hypothalamus controls the setpoint of β -cells regarding the quantity of insulin being released after a certain glucose load (Steffens *et al.* 1991). Thus, our data could be explained by a shift in central nervous system control from a predominant sympathetic nervous system tone through VMH neuronal activity to an increased parasympathetic tone by the chronic treatment with the D_2 -dopaminergic agonist.

Peripheral action of bromocriptine on adrenomedullary secretion could also have contributed to the lower hyperglycemic response and to the increased insulin secretion after intravenous injection of 2DG in the bromocriptine-treated rats. The presence of dopamine D_2 receptors has been shown in the adrenal medulla and administration of D_2 receptor agonists reduced catecholamine secretion induced by nicotine activation (Artalejo *et al.* 1985, Gonzales *et al.* 1986). Therefore, the D_2 agonist inhibition of catecholamine secretion by the adrenal medulla should have facilitated the higher insulin response in bromocriptine-treated rats submitted to i.v. injection of 2DG.

The group of rats treated with vehicle showed the expected fast prolactin release in response to neuroglucopenic stress induced by 2DG injection as observed by others (Woolf *et al.* 1977, Okajima *et al.* 1980). The group of rats treated with the D_2 receptor agonist had their stress-induced prolactin secretion completely blocked, indicating a strong inhibition by treatment with bromocriptine. These results are similar to those observed by Okajima *et al.* (1980) using apomorphine, a dopaminergic agonist, to block neurocytoglucopenia-induced prolactin release. Therefore, our results provide further evidence that neuroglucopenia is also a potent stimulus for prolactin release, under D_2 -dopaminergic mechanism control; this can be compared with other models of stress commonly chosen to study the regulation of prolactin secretion in rats (Reis *et al.* 1998).

Studies from our laboratory have recently shown that hyperprolactinemia is related to changes in carbohydrate metabolism in several models of stress (Reis *et al.* 1994, 1996a,b, 1998). However, we do not believe that the inhibition of prolactin secretion by bromocriptine has contributed in any way to the increased hyperinsulinemic response since prolactin has been shown to increase β -cell proliferation, insulin synthesis and glucose-stimulated insulin secretion in rats (Reis *et al.* 1997, Sorenson & Brelje 1997).

Finally, we conclude that chronic treatment with the D_2 receptor agonist determines a shift in control of neuroglucopenia from a predominant sympathetic tone to a parasympathetic tone, accounting for the lower hyperglycemic and strong insulin responses following 2DG infusion.

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