J. Pharm. Pharmacol. 1982, 34: 377–380
Received November 11, 1981

0022–3573/82/060377–04 $02.50/0

Effect of reserpinization on insulin secretion in the rat

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Reserpine-induced supersensitivity to the insulin-releasing action of a β-adrenergic agonist, isoprenaline, and of glucose was studied in vivo and in vitro. The subcutaneous injection of rats with reserpine (0·05 to 10 mg kg^{-1}) enhanced the action of isoprenaline on insulin secretion. ED50 of isoprenaline for insulin secretion was changed little after reserpinization, whereas maximum effect of the β-agonist was enhanced by pretreating rats with reserpine. Glucose-stimulated insulin secretion was also enhanced in the reserpinized rats. Pancreases isolated from the reserpinized rats secreted more insulin in response to phentolamine in the presence of glucose and isoprenaline. These results suggest that the supersensitivity in insulin secretion induced by reserpine may be non-specific.

In the course of an investigation of regulatory mechanisms of insulin secretion in the rat, we noticed that reserpinization of the rats augmented the hyperinsulinaemic effect of isoprenaline, a typical β-adrenergic agonist. Although the major physiological secretagogues for insulin may be glucose and amino acids, β-adrenoceptor-mediated secretion of insulin has been studied in vivo and in vitro (Malaisse et al 1967; Katada & Ui 1977). The pancreatic secretion of insulin is promoted by stimulation of β-adrenoceptors and inhibited by stimulation of α-adrenoceptors in the islets (Montague & Howell 1975). Meisheri et al (1979) recently reviewed 'reserpine-induced supersensitivity to the cardiac effects of agonists'. Results to be presented in this paper suggest that there is also an increase in sensitivity of insulin secretion to secretagogues in reserpinized rats.

MATERIALS AND METHODS

Male rats, 200 to 250 g, of the Wistar-derived strain (Donryu) were used. Reserpinization was carried out in rats by subcutaneous injection of the drug (0·05 to 10 mg kg^{-1} day^{-1}) for 1 to 6 days. Immunoreactive insulin was determined by the method of Herbert et al (1965). The amount of insulin was expressed as μunit ml^{-1} of plasma. Isoprenaline (200 μg kg^{-1}) was injected s.c. into rats. In some experiments, glucose as a 30% solution was injected i.p. (1 mg/100 g). In the 'perfusion experiment', the pancreas was isolated and perfused at a flow rate of 2·0 ml min^{-1} by the method of Grodsky et al (1963) with minor modifications (Toyota et al 1975). The effluent was continuously collected by a fraction collector from the 20th to 45th min, over successive periods of 1 min each, and the insulin concentration in the effluent was determined by the same method (Herbert et al 1965).

The drugs used were: reserpine (Nakarai Chemicals, Ltd), isoprenaline (Sigma Chemical Co.), and phentolamine (Regitine, Ciba-Geigy, Japan). Other reagents were of analytical grade.

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RESULTS

Effect of reserpinization of rats on action of isoprenaline to increase the plasma concentration of insulin

Fig. 1 shows that isoprenaline increased the plasma level of insulin more in reserpinized rats (10 mg kg^{-1} day^{-1} for 6 days) compared with control rats. Since the dose of reserpine used was extremely large,

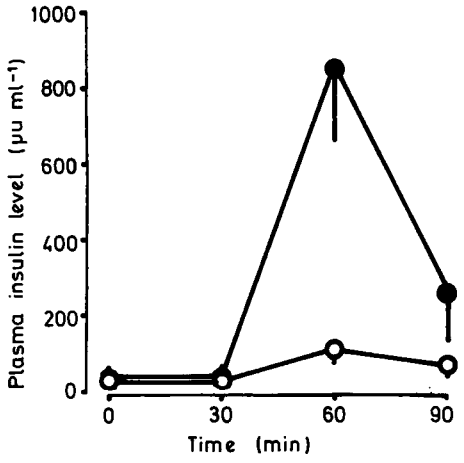

FIG. 1. Effect of subcutaneous injection of isoprenaline (200 μg kg^{-1}) on the plasma level of insulin in normal (○—○) and reserpinized (10 mg kg^{-1} for 6 days ●—●) rats. Mean ± s.e.m. from 4 observations. Isoprenaline was injected at 30 min.

another experiment with a lower dose of reserpine has been done (Fig. 2). Three mg kg^{-1} of reserpine was injected once into rats 24 h before the experiment. In the rats thus treated, isoprenaline elevated the plasma concentration of insulin about two-fold compared with control rats.

Basal plasma level of insulin in reserpinized rats

Injection of reserpine into rats resulted in about 50 to 100% increase in plasma level of insulin as shown in Table 1. The plasma concentrations of insulin were determined 24 h after the last injection of reserpine. There was not a large difference in the effectiveness of reserpine between rats treated with the low (3 mg kg^{-1} for 1 day) and those treated with the high (10 mg kg^{-1} for 6 days) dose of the drug.

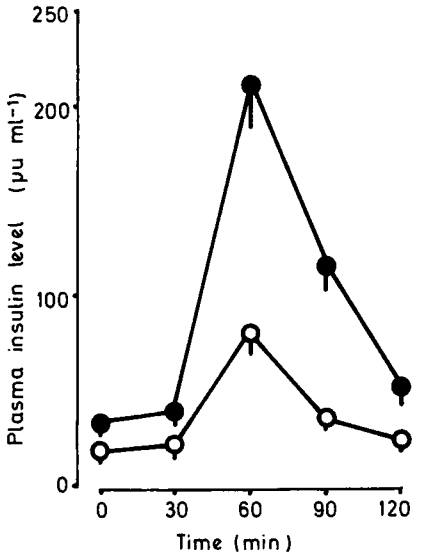

FIG. 2. Effect of subcutaneous injection of isoprenaline (200 μg kg^{-1}) on the plasma level of insulin in normal (○—○) and reserpinized (3 mg kg^{-1} for 1 day, ●—●) rats. See for other conditions the legend for Fig. 1.

Dose of reserpine and duration of effective period of reserpine treatment

The dose-response relationship for reserpine treatment is shown in Fig. 3. 24 h after injection of reserpine, isoprenaline was given i.p. The plasma concentration of insulin 30 min after the isoprenaline was plotted as a percentage of control values. Reserpine at as low as 0·05 mg kg^{-1} enhanced isoprenaline-stimulated insulin secretion to about 120% of control. At doses from 0·1 to 15 mg kg^{-1}, the drug increased the action of isoprenaline.

In Fig. 4, the duration of the effect of reserpine at 1 mg kg^{-1} is shown. At only 3 h after injection of reserpine, isoprenaline-stimulated insulin release was enhanced to about 240% of control. Although the degree of enhancement was gradually reduced, this effect of reserpine continued for at least 5 days.

Table 1. Effect of treatment of rats with reserpine on plasma level of insulin. Reserpine was injected s.c. at a dose indicated. The plasma concentration of insulin were determined 24 h after the last injection of reserpine.

Conditions (Reserpine)	Plasma insulin level (μu ml^{-1})	Difference
None	13·3 ± 1·7 (4)	
3 mg kg^{-1} for 1 day	29·6 ± 3·8 (4)	+16·3**
None	18·1 ± 2·1 (4)	
10 mg kg^{-1} for 6 days	26·2 ± 2·2 (4)	+8·1*

* $P < 0.05$ and ** $P < 0.01$ as compared to none. Number of animals given in parentheses.

Dose-dependent increases in the plasma concentration of insulin by isoprenaline in control and reserpinized rats are shown in Fig. 5. The concentration of isoprenaline causing the half-maximal increase, ED50, in reserpinized rats was essentially the same as that in control rats. In contrast, the maximum response to isoprenaline in control rats was smaller than that in reserpinized rats.

Effect of glucose on plasma insulin level in reserpinized rats

The effect of glucose on the plasma level of insulin was compared between the normal and reserpinized

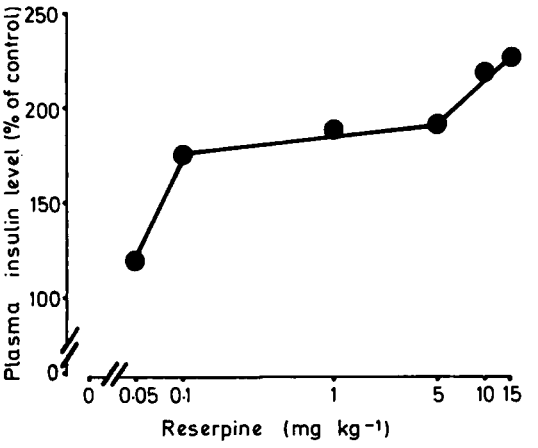

FIG. 3. Dose-response relationship for reserpine treatment. At 24 h after injection of reserpine at each dose indicated, isoprenaline (200 μg kg^{-1}) was given i.p. to rats. The plasma concentrations of insulin 30 min after isoprenaline injection were plotted as a percentage of control value. Each plot was the average of from 2 to 4 observations.

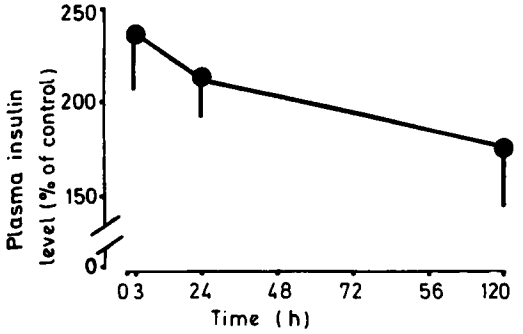

FIG. 4. The duration of effect of reserpine. 1 mg kg^{-1} of reserpine was injected at 0 time. After the injection of reserpine, the effects of isoprenaline (200 μg kg^{-1}) on plasma insulin level were examined at the 3rd, 24th, and 120th h. Plasma insulin levels 30 min after each isoprenaline injection were plotted as mean ± s.e.m. from 5 observations. Ordinate: Plasma insulin level (% of control). Abscissa: Time (h).

rats. After injections (i.p.) of glucose at a dose of 300 mg/100 g, there was a larger increase in the plasma insulin level in the reserpinized rats. The net increment (difference between initial and peak levels) after glucose in the plasma insulin level of the reserpinized rats was 48 ± 8·1 μu ml^{-1} of plasma, whereas the control value was 23 ± 4·7 μu ml^{-1}.

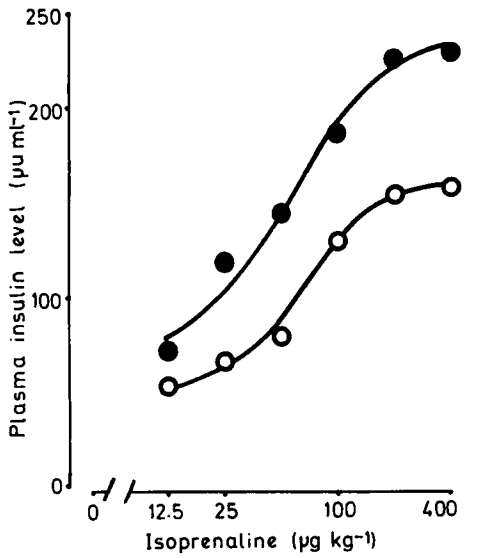

FIG. 5. Dose-dependent curves for isoprenaline-induced increase in the plasma concentration of insulin. Rats were injected i.p. with 1 mg kg^{-1} of reserpine (○—○) or 0·3 ml of saline as control (●—●). 24 h after the injection, isoprenaline at a dose indicated in Fig. was injected. Each plot illustrated was the average of from 2 to 4 observations.

Effect of isoprenaline on insulin secretory response in perfused pancreases from reserpinized rats

Pancreases isolated from normal and reserpinized rats were perfused with perfusate containing 240 mg/100 ml of glucose. At this concentration of glucose, a 15 to 20 min perfusion was required before the rate of insulin release became almost constant. Isoprenaline (0·5 μM) added to the perfusate at the 25th min had a very small transient effect on the rate of insulin secretion. There was no difference between the response of pancreases from normal and those from reserpinized rats. However, when phentolamine was added to the perfusate, the rate of insulin secretion in pancreases from reserpinized rats was significantly higher than that in control pancreases.

DISCUSSION

We have shown in this study that pretreatment with reserpine effects changes in the characterisitics of the rat insulin secretion system. This was shown by the increase in the response of insulin secretion to isoprenaline, and to glucose in vivo, and to phentolamine in the presence of glucose and isoprenaline in vitro. Those results suggest that the supersensitivity may be non-specific, and that reserpine-induced supersensitivity, not only to the cardiac actions of various agonists (Meisheri et al 1979) but also to the pancreatic effects of secretagogues, could be induced in the rats.

Gibson & Pollock (1975) reported that a single dose of reserpine quickly raised plasma corticosterone levels, which almost doubled in the 1st hour of treatment, after which the levels fell slowly, and that the rise of corticosteroids was involved in the supersensitivity produced in the rat anococcygeus muscle by reserpine. The fact that the supersensitivity of insulin secretion to isoprenaline was quickly observed in the 3rd hour of reserpine treatment (Fig. 4) seems to suggest the involvement of corticosteroids in the rapid supersensitivity. However, whether the prolonged supersensitivity such as that seen on the 5th day after reserpine treatment is also caused by the rise of corticosteroids which lasted for less than a day (Gibson & Pollock 1975) is doubtful. Two or more mechanisms might be involved.

McNeill (1969) suggested that reserpine-induced supersensitivity of the cardiac response to catecholamines may be connected with the effect of reserpine on the calcium permeability. Reserpine is now thought to disturb normal calcium homeostasis. Calcium ions are involved in the insulin secretion mechanism enhanced by sensitization with pertussis

(Katada & Ui 1979). However, the actual involvement of calcium ions in the reserpine-induced hypersensitivity in insulin secretion is not clear.

The large doses of reserpine, such as that used in Fig. 1, produced profound sedation and prostration with a marked decrease in food intake. Starvation inhibits insulin release in response to various secretagogues. Feldman & Lebovitz (1973) have shown that pancreatic catecholamines may play a role in the decreased insulin secretion of the fasting state. Reserpine is noted for producing depletion of catecholamines. Depletion of catecholamines may also increase the number of available adrenoceptors. The maximum response to isoprenaline in reserpinized rats was higher than that in control rats, whereas ED50 in both was essentially the same (Fig. 5). Therefore, it could be anticipated that the effects of reserpine on insulin secretion, even at a low dose,

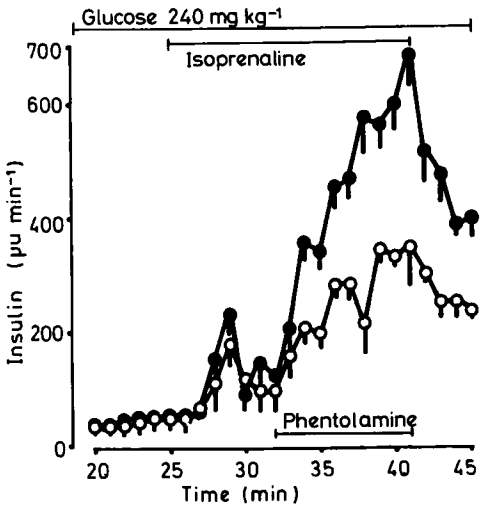

FIG. 6. Effect of phentolamine (42 μM) on the rate of insulin release from the pancreases isolated from normal (○—○) and reserpinized (●—●) rats in the presence of 0·5 μM of isoprenaline and 240 mg% of glucose. Each point represents the mean of insulin secretion rate with s.e.m. as a vertical line (number of observations, 4).

would be due to depletion of catecholamines which inhibit insulin secretion, and that catecholamine depletion in pancreas (Jaim-Etcheverry & Zieher 1968) would augment the hyperinsulinaemic effect of various secretagogues even in the perfused pancreas from reserpinized rats. As shown in Fig. 6, however, neither glucose nor isoprenaline stimulated insulin secretion more in the perfused pancreas from reserpinized rats than in those from normal rats. The result indicates that the effect of reserpine on glucose- and isoprenaline-induced hyperinsulinaemia may reflect, in part, decreased clearance of insulin from the blood.

The addition of phentolamine to the perfusate containing glucose and isoprenaline, stimulated insulin secretion more in the pancreas from reserpinized rats (Fig. 6). This may not be the result of an α-adrenoceptor blocking action but to glucose in the perfusate, because Cryer et al (1971) reported that phentoloamine increases the response of the β-cell to glucose although it has no effect on the basal rate of insulin release. However, more work is needed to explain clearly the discrepancy between the in vivo and in vitro observations.

Acknowledgements

We are greatly indebted to Dr M. Ui for his guidance and encouragement during this study. We also thank Miss M. Kataoka for her technical assistance.

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