Inhibition of Insulin Release from Rat Pancreatic Islets by Drugs that Are Analogues of Dopamine

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SUMMARY

Various synthetic dopamine (DA) analogues have been shown to produce glucose intolerance and inhibit the compensatory increase in serum insulin during an oral glucose tolerance test (OGTT). To investigate the possibility that there is a direct action of dopamine analogues to inhibit glucose-stimulated insulin release from the endocrine pancreas, the following compounds were compared with the effects of epinephrine (EPI) on isolated rat pancreatic islets: apomorphine (APO), pergolide, lergotrile, TL-99 (2-dimethylamino-6,7-dihydroxytetralin), and RDS-127 (2-di-n-propylamino-4,7-dimethoxyindane). EPI, TL-99, and pergolide inhibited insulin release in a concentration-dependent fashion (10⁻⁷–10⁻⁵ M), whereas lergotrile inhibited at 10⁻⁵ M but not at 10⁻⁶ M. RDS-127 and APO were ineffective at 10⁻⁵ M, but produced a greater than 50% inhibition at 2 x 10⁻⁶ M. The potencies of the DA analogues fell into two groups: compounds that are approximately as active as EPI (e.g., TL-99 and pergolide) or compounds that are relatively inactive (e.g., APO, lergotrile, and RDS-127). The inhibitory actions of EPI, TL-99, and pergolide were blocked by the α-adrenergic receptor antagonist yohimbine, whereas the DA receptor antagonist, sulpride, had no effect, suggesting an action initiated at α₂-adrenergic receptors. Drugs from both groups produced marked glucose intolerance and inhibited the compensatory increase in insulin during an OGTT. Adrenomedullation blocked the glucose intolerance and inhibition of insulin release caused by RDS-127, whereas these effects of TL-99 were not attenuated. These data, obtained in vitro and in vivo, show that selective dopamine agonists, such as TL-99, directly inhibit the release of insulin from the endocrine pancreas. DIABETES 1984; 33:888-93.
ured during an oral glucose tolerance test (OGTT) in rats. It is possible that TL-99, by interacting with α-adrenergic receptors in pancreatic islets, could cause these effects through an inhibition of insulin release from the pancreas. On the other hand, there is evidence that the hyperglycemic effects of apomorphine and RDS-127 result from sympathoadrenal activation, permitting an epinephrine-induced increase in blood glucose. It remains possible, however, that the effects of apomorphine and RDS-127 are, at least in part, the result of a direct inhibition of insulin release from pancreatic islets.

The potencies with which various DA analogues directly inhibit glucose-stimulated insulin release in isolated pancreatic islets have not been previously determined. Those analogues that produce hyperglycemia by a direct action on the endocrine pancreas would be expected to have a potency for inhibiting insulin release from isolated pancreatic islets similar to that of epinephrine. Analogues producing hyperglycemia by acting on DA receptors in the central nervous system should be less potent in their action to inhibit hormone release from isolated islets. In the present study, we determined the relative potencies of various DA analogues and epinephrine for inhibition of glucose-stimulated insulin release from rat pancreatic islets. See Figure 1 for the chemical structures of the DA analogues used in this study. The possible involvement of dopaminergic and/or adrenergic receptors in the potent inhibition of in vitro insulin release by certain DA analogues was assessed using the antagonists sulpiride and yohimbine. In addition, we examined whether the adrenal medulla is required for the in vivo inhibition of insulin release caused by the administration of RDS-127 and TL-99, these compounds being representative of relatively specific and nonspecific dopamine agonists, respectively. The results in this report provide additional evidence that DA-like drugs can produce hyperglycemia by two different mechanisms.

MATERIALS AND METHODS

Animals. Male, Sprague-Dawley rats (200–350 g) were subjected to a 12 h/12 h light/dark schedule with the lights on from 0730 h to 1930 h. Standard rat chow and water were available ad libitum. Room temperature was 21 ± 2°C.

Isolated islets. Islets of Langerhans were isolated by collagenase digestion of the pancreas as described by Lacy and Kostianovsky. Groups of 10 islets collected in 0.1-ml volume were added to 13 × 100-mm, siliconized glass tubes. Krebs-Ringer bicarbonate buffer (0.8 ml) containing 2.0 mg/ml bovine serum albumin and low glucose (0.75 mg/ml) was added to all tubes so that a final volume of 1.0 ml contained 0.6 mg/ml glucose. Dopamine receptor agonists (2.4 × 10−6 to 10−5 M) or receptor antagonists (10−6 to 10−4 M) were added in a total volume of 0.1 ml to obtain the final concentrations as indicated; control tubes received 0.1 ml vehicle (0.9% NaCl). Dopamine receptor agonists were added to the islets and incubated at 37°C for 10 min before high glucose stimulation. Antagonists (or vehicle), when used, were added to islets 5 min before addition of the agonists. After this pretreatment schedule, the tubes were gently centrifuged to pellet the islets and the supernatant was aspirated and discarded. Test compounds (i.e., antagonists and/or agonists) were added back to the tubes in 0.9 ml buffer solution that contained high glucose (3.0 mg/ml).

Trasyrol (500 KIU/ml; FBA Pharmaceuticals, Inc., New York, New York) was introduced into all tubes to inhibit proteolytic degradation of insulin. The tubes were flushed with 95% O2/5% CO2 and capped. After gentle shaking in a water bath at 37°C for 45 min, the tubes were removed and the islets pelleted by gentle centrifugation. The supernatant was obtained and frozen (−20°C) until assay for immunoreactive insulin (IRI). Included in each experiment were two groups of control islets that were not treated with antagonists or agonists, but were exposed only to 0.6 mg/ml glucose (low-glucose control) or 3.0 mg/ml glucose (high-glucose control) for 45 min. These control islets were handled in exactly the same manner as islets that were treated with antagonists and agonists, except that additions of vehicle were made instead of solutions containing drugs. Data were calculated as ng IRI/islet/45 min incubation. Because of the usual day-to-day variation in glucose-stimulated insulin release from isolated pancreatic islets, the data are expressed as percent of high-glucose controls to facilitate the statistical comparison of drug effects.

OGTTs. Rats were fasted for 18 ± 1 h before these experiments, with water still available ad libitum. For repetitive withdrawal of blood samples, a catheter was placed in the jugular vein the afternoon before experimentation (1400–1600 h). One end of the catheter (0.3-mm i.d. silastic tubing) was inserted through the right jugular vein into the atrium of the heart of rats anesthetized with pentobarbital sodium (40 mg/kg, i.p.), while the other end was exteriorized behind the head. Catheters were flushed with heparin (100 U/ml) to maintain patency. Blood samples were obtained from conscious, unrestrained animals.

Adrenomedullation was completed under pentobarbital sodium anesthesia (40 mg/kg, i.p.) through a midline abdominal laparotomy performed 1 wk before experimentation. Saline (vehicle control), TL-99 (3.6 μmol/kg), or RDS-127...
The following compounds were obtained as generous gifts from the indicated companies: TL-99 (2-di-methylamino-6,7-dimethoxyindane hydrochloride), Merck, Sharp and Dohme (West Point, Pennsylvania); lergotrile mesylate and pergolide mesylate, Eli Lilly and Company (Indianapolis, Indiana); sulpiride injectable, Delegrange Laboratories (Paris, France); and yohimbine hydrochloride, K and K Laboratories, Inc. (Plainview, New York).

RESULTS

Pancreatic islets. Figure 2 shows the concentration-response relationship of epinephrine and various DA analogues to inhibit in vitro glucose-stimulated insulin release. Epinephrine and TL-99 inhibited insulin release >50% at concentrations of $10^{-7}$ and $10^{-6}$ M. At a concentration of $10^{-5}$ M, epinephrine and TL-99 had profound effects on insulin release, the effects of pergolide were significantly less than those of epinephrine, and lergotrile had no significant inhibitory effects. At $10^{-5}$ M, pergolide had a maximal inhibitory effect, lergotrile also significantly inhibited release, but both APO and RDS-127 were inactive. The inhibitory effects of APO and RDS-127 were not detected until very high concentrations ($2 \times 10^{-4}$ M) were used.

Figure 3 shows the effects of the antagonists yohimbine ($10^{-5}$ M) and sulpiride ($10^{-8}$ M) on the inhibitory effects of $10^{-6}$ M concentrations of epinephrine, TL-99, or pergolide. The $\alpha_2$-adrenergic receptor antagonist, yohimbine, blocked the inhibitory effects of epinephrine, TL-99, and pergolide, whereas the DA receptor antagonist, sulpiride, had no effect. The antagonists alone, yohimbine ($10^{-5}$ M) or sulpiride ($10^{-8}$ M), had no significant effect on insulin release stimulated by high glucose (97 ± 5% and 101 ± 4% of control, respectively, N = 3). The antagonists were not used with APO or RDS-127 due to the low potency of these agonists for inhibition of glucose-stimulated insulin release from pancreatic islets.

Insulin assay. Immunoreactive insulin (IRI) was measured radioimmunologically by the method of Morgan and Lazard.21 Rat insulin (Novo, Copenhagen) was used as the standard and guinea pig antiserum to porcine insulin (Dr. P. Wright) was the antibody source. [125]I-insulin was prepared by the Diabetes Research Center Core Unit at the University of Iowa. Radiochemical purity of [125]I-insulin was verified by a high-performance liquid chromatographic technique.25

Statistics. Data were analyzed by one-way analysis of variance using Duncan’s new multiple-range test to detect treatment differences.23 Data expressed as percent of control were transformed to the corresponding log values before statistical analysis. The 0.05 level of probability was chosen as the criterion of statistical significance. Values indicated in the text or figures represent the mean ± SEM.
FIGURE 4. The effects of sham operation or adrenodemedullation on the glucose intolerance produced by RDS-127 (left panel) and TL-99 (right panel) during an OGTT. RDS-127 (8.4 μmol/kg), TL-99 (3.6 μmol/kg), or saline was administered subcutaneously immediately after the -30 min blood sample. Values represent the change in serum glucose from the pre-experiment (-30 min) value. The basal glucose concentrations (-30 min) for the sham and adrenodemedullated animals were 89 ± 3 (N = 23) and 87 ± 2 mg/dl (N = 26), respectively. The shaded region represents the mean ± SEM (N = 19-20) of the saline (vehicle control) responses. An asterisk (*) indicates that the value is statistically different from the vehicle control response, P < 0.05.

OGTT. To demonstrate whether RDS-127 and TL-99 exerted direct or indirect actions to alter β-cell function in vivo, the effects of adrenodemedullation on the ability of these dopamine analogues to inhibit insulin release during an OGTT were determined. If the inhibitory actions of RDS-127 or TL-99 were attenuated by adrenodemedullation, then adrenal medullary secretions could be implicated in contributing to the ability of these drugs to impair β-cell function.

There were no differences between sham-operated or adrenodemedullated animals in serum glucose or serum insulin concentrations during an OGTT. Therefore, the serum glucose and insulin responses for saline-treated (vehicle control) animals were pooled from sham-operated and adrenodemedullated animals; these control values are shown in Figures 4 and 5. Adrenodemedullation blocked the glucose intolerance produced by RDS-127 (Figure 4, left panel), whereas adrenodemedullation did not attenuate the glucose intolerance produced by TL-99 (Figure 4, right panel). Adrenodemedullation significantly attenuated the inhibition by RDS-127 of the compensatory increase in serum insulin caused by glucose administration (Figure 5, left panel), whereas the inhibitory effects of TL-99 on glucose tolerance were not affected (Figure 5, right panel). Figure 6 shows the effect of adrenodemedullation on the changes in the insulin/glucose ratio produced by RDS-127 or TL-99 30 min after an oral glucose load, the insulin/glucose ratio being an index of β-cell function. Both RDS-127 and TL-99 significantly inhibited the normal response of the β-cells to an oral glucose load. While adrenodemedullation prevented the effects of RDS-127, adrenodemedullation had no effect on the inhibitory actions of TL-99.

DISCUSSION

The present study shows that there are wide differences in the potencies required for dopamine analogues to inhibit glucose-stimulated insulin release from rat pancreatic islets. Pergolide and TL-99 have potencies for inhibition of glucose-stimulated insulin release similar to epinephrine, while RDS-127 and apomorphine are much less potent when tested in the pancreatic islet system. Previously, it has been shown that all of these drugs will produce hyperglycemia after doses that produce similar central dopaminergic stimulant effects. Based on potency to inhibit insulin secretion from pancreatic islets, the results suggest that TL-99 and pergolide could produce alterations in blood glucose by directly altering insulin secretion in an epinephrine-like manner, whereas apomorphine and RDS-127 may lack sufficient potency for a direct action in pancreatic islets.

It is clear from the data obtained from OGTTs that the effects of RDS-127 and TL-99 to produce abnormal glucose tolerance and inhibit in vivo insulin release are brought about by different mechanisms. The presence of the adrenal medulla is necessary for the inhibitory action of RDS-127 on pancreatic function during an OGTT, but not for the effect
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that phentolamine, an a-adrenergic receptor antagonist, co-workers 16 have suggested that a-receptors of the a 2-...ions of the DA receptor antagonists haloperidol and in vitro through a-adrenergic and not through DA receptor-mediated processes. Itoh and co-workers 17 demonstrated blocks the inhibitory action of DA, whereas high concentrations of the DA analogues studied here to inhibit glucose-stimulated insulin release in vivo. Apomorphine, RDS-127, and lergotrile have been well characterized and are considered to have much less central a- and b-adrenergic receptor agonist action than does dopamine itself. The ability of TL-99 and the inability of RDS-127 to interact with a 2-adrenergic receptors within peripheral tissues other than the pancreas has been well documented. 19,20-23 Pergolide is known to interact with peripheral a-adrenergic receptors. 13 Thus, in considering the hyperglycemic action of dopamine analogues, it is consistent with results obtained in other biologic systems to suggest that analogues of DA, such as TL-99, act to directly inhibit insulin release in pancreatic islets through a 2-adrenergic receptors, whereas RDS-127, apomorphine, and, probably, lergotrile activate the sympa...the structural requirements for adrenergic receptor-mediated inhibition of insulin release.

The apparent differences in the mechanism of the hyperglycemic effects of the dopamine analogues studied here are consistent with differences among these drugs observed in other biologic test systems. Apomorphine, RDS-127, and lergotrile have been well characterized and are considered to be relatively specific for interacting with dopamine receptors in the CNS. 18-20,23 These dopamine analogues appear to...Structures, support the results obtained with DA by Itoh and co-workers. Although the structural requirements that allow...of TL-99. Previously, it has been shown that the adrenal gland is required for the action of RDS-127, apomorphine, and lergotrile to produce hyperglycemia in unchallenged rats. Together with the results presented here, it appears that RDS-127, lergotrile, and apomorphine alter b-cell function in vivo through adrenal medullary secretions, but that TL-99 acts as a direct inhibitor of insulin release. Although the effects of pergolide have not been fully evaluated in vivo, its high potency to inhibit insulin release in vitro suggests that its action in vivo could be a direct one (i.e., similar to that of TL-99).

Previous work suggests that DA reduces insulin release in vitro through a-adrenergic and not through DA receptor-mediated processes. Itoh and co-workers 17 demonstrated that phentolamine, an a-adrenergic receptor antagonist, blocks the inhibitory action of DA, whereas high concentrations of the DA receptor antagonists haloperidol and pimozide are ineffective. Recently, Nakaki and co-workers 24 have suggested that a-receptors of the a-adrenergic subtype are present in pancreatic islets, since yohimbine (10^-5 M) was shown to completely block the inhibitory action of the a-adrenergic receptor agonist epinephrine. In the present report, yohimbine (10^-5 M) was also shown to attenuate the inhibitory effects of epinephrine, TL-99, and pergolide (all at 10^-4 M). In contrast, the DA receptor antagonist sulpiride, which is a very weak antagonist of a-adrenergic and serotonin receptors, 26 had no effect on the inhibitory actions of these three agents. The dose of sulpiride used here (10^-4 M) has been established to block DA receptor-mediated actions in vitro. 27 Therefore, our results, obtained using synthetic DA analogues of diverse chemical structures, support the results obtained with DA by Itoh and co-workers. Although the structural requirements that allow a DA analogue to directly modify b-cell function are not apparent (refer to Figure 1), it is clear that a catecholamine component is not required (e.g., pergolide). These conclusions are different from those derived by Feldman and co-workers, 21 who found that phenylethylamine derivatives require hydroxyl groups on the aromatic ring to have a significant action to inhibit glucose-stimulated insulin release in vitro. Further work will be necessary to completely elucidate the structural requirements for adrenergic receptor-mediated inhibition of insulin release.
lacking potency to inhibit insulin secretion in vitro, act in vivo by activating central dopaminergic receptors, which results in the release of epinephrine from the adrenal medulla.\(^{20}\) In contrast to this indirect mechanism, TL-99 and, probably, pergolide, through \(\alpha\)-adrenergic actions, directly inhibit glucose-stimulated insulin release by interacting at receptors located in pancreatic islets.

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REFERENCES


