Inhibitory Effects of Rimorphin and Dynorphin on Insulin Secretion from the Isolated, Perfused Rat Pancreas

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ISHIZUKA, J., TOYOTA, T., ONO, T., SAKAI, M., YANAIHARA, C. and YANAIHARA, N. Inhibitory Effects of Rimorphin and Dynorphin on Insulin Secretion from the Isolated, Perfused Rat Pancreas. Tohoku J. exp. Med., 1986, 150(1), 17-24 — In order to settle the question about whether or not opioid peptides stimulate or inhibit insulin secretion, we studied effects of rimorphin and dynorphin, two members of the preproenkephalin B group, on glucose-induced insulin secretion in the isolated, perfused rat pancreas. These peptides (3.95 x 10^-8 M), like morphine (3.95 x 10^-8 M), significantly inhibited the glucose-induced insulin secretion. The inhibitory effect of rimorphin was attenuated by naloxone (1.2 x 10^-6 M) and phentolamine (10^-6 M), suggesting an involvement of adrenergic alpha receptors in the inhibition of glucose-induced insulin secretion mediated through specific opiate receptors. Rimorphin also inhibited glucose-induced insulin secretion even in the cysteamine-treated rat pancreas from which somatostatin had been depleted. Thus, somatostatin does not appear to play a major regulatory role in the insulin secretion in the pancreas. —— insulin secretion ; dynorphin ; rimorphin ; perfusion of the isolated rat pancreas

The fact that infusion of beta-endorphin diminished the glucagon and insulin responses to epinephrine indicates a possible role for the endogenous opiates in modulating the glucoregulatory response to the stress (El-Tayeb et al. 1985). It has been reported that endorphins and enkephalins are released in a variety of stress situations, including exercise (Fraioli et al. 1980) and hypoglycemia (Nakao et al. 1979). A question arises as to whether opioid peptides act on glucose metabolism via pancreatic hormones, i.e., insulin and glucagon. Pancreatic extracts have been shown to contain peptides eluting with similar characteristics to the enkephalins (Bruni et al. 1979). The existence of opioid peptides including enkephalins in the pancreas may support the concept that these peptides act as a physiologic regulator of pancreatic endocrine secretion. However, there have

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been discrepancies in the opinion on effects of opioid peptides on insulin secretion, i.e., they stimulate the secretion of insulin (Ipp et al. 1978, 1982; Green et al. 1980, 1983; Hermansen 1983; Scala-Guenot and McIntosh 1985) or inhibit it (Kanter et al. 1980; Rudman et al. 1983). The discrepancies of the results have caused much controversy, being ascribed to the methodological differences (Giugliano 1984). From a view point of neuroendocrine-regulation of insulin secretion, opioid peptides coexisting with catecholamines (Shimosegawa et al. 1983) are presumed to possess an inhibitory action on insulin. Such an inhibition caused by direct action on specific opiate receptors may be modified by somatostatin (paracrine regulation) or catecholamines. We investigated the effects of rimorphin and dynorphin on glucose-induced insulin secretion using the isolated, perfused rat pancreas. Rimorphin, belonging to preproenkephalin B group, consists of 13 amino acids which contains leu-enkephalin at the N-terminus. Dynorphin consists of 17 amino acids which contains leu-enkephalin at the N-terminus.

**Materials and Methods**

Male Wistar rats weighing 200-250 g were used. They were allowed to have chows and water ad libitum till just before the perfusion experiments. Overnight-fasted rats were anesthetized by intraperitoneal injection of pentobarbital sodium (45 mg/kg) and the pancreas associated with duodenum was isolated according to the procedure described by Grodsky et al. (Grodsky et al. 1963) with some modification (Toyota et al. 1975, 1978). The perfusate consisted of Krebs Ringer bicarbonate buffer containing 4% Dextran T 70 (Pharmacia Fine Chemicals, Tokyo) and 0.5% bovine serum albumin (Sigma Chemical Co., St. Louis, MO, USA) gassed with 95% O₂ and 5% CO₂ to pH 7.4. The pancreas was perfused with 75 mg/100 ml glucose of equilibration for the first 20 min at a rate of 2.5 ml per min and then with 150 mg/100 ml glucose throughout the 35 min perfusion. The pancreas was perfused with rimorphin (3.95×10⁻⁸ M) or dynorphin (3.95×10⁻⁸ M) for the midst 10 min at 15 min after the start of 150 mg/100 ml glucose infusion. The complete effluent was collected from the cannula in the portal vein at 1 min intervals in chilled tubes and frozen for storage at -20°C until assay.

Insulin was measured by radioimmunoassay of a double antibody system (microsepharose method, Shionogi, Osaka).

Two kinds of experiments were conducted to test the hypothesis that rimorphin action on the pancreatic beta cells may be mediated by the regulatory mechanism of somatostatin and adrenergic hormones; first, the pancreata were perfused with phenolamine (10⁻⁶ M), propranolol (7.5×10⁻⁴ M or naloxone (3.95×10⁻⁸ M, 1.2×10⁻⁶ M) during the infusion of rimorphin, and secondly, 300 mg/kg cytemine hydrochloride was given orally to 4 male rats in order to deplete somatostatin from the pancreatic islets. The pancreata were isolated from the 4 rats next day after the administration of cytemine, and then were perfused with rimorphin in the presence of 150 mg/100 ml glucose as described previously. The cytemine-treated pancreata were stained immunohistochemically with anti-somatostatin antibody.

Total insulin response to 150 mg/100 ml glucose in the presence and absence of interfering substances was calculated as an integrated insulin response (IIR) by integration of the area under the individual perfusion profiles, and differences in IIR under both conditions were deemed as the effect of the interfering substances.

The following substances were used: Dynorphin, rimorphin, naloxone (Sigma, St.
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Louis, Mo, USA), morphine hydrochloride (Wako, Tokyo), cysteamine hydrochloride (Wako, Tokyo) and phentolamine (Ciba, Basel, Switzerland), and propranolol hydrochloride (Sigma). Rimorphin was synthesized by Yanaihara. Dynorphin was kindly given by Dr. Tachibana (1982).

All results were expressed as mean±s.e. and statistical analysis was performed using Student’s t-test for unpaired data.

RESULTS

Effects of dynorphin, rimorphin and morphine on insulin release from the perfused pancreas

Insulin secretion in response to 150 mg/100 ml of glucose was suppressed by dynorphin or rimorphin (3.95×10^{-8} M), as shown in Figs. 1 and 2. Integrated insulin responses (IIRs) to glucose were decreased by dynorphin and rimorphin by 44.2±12.3 ng·min^{-1}·ml^{-1} and 21.2±6.3 ng·min^{-1}·ml^{-1}, respectively (Table 1). Morphine (3.95×10^{-8} M) also inhibited glucose-induced insulin secretion (Fig. 3) and the decrease in IIR was 77.9±14.0 ng·min^{-1}·ml^{-1}. The inhibitory effect of rimorphin (3.95×10^{-8} M) on glucose-induced insulin secretion was antagonized by naloxone (1.2×10^{-6} M), whereby the decrease in IIR was 1.8±3.46 ng·min^{-1}·ml^{-1} (Table 1). On the other hand, naloxone at a concentration of 3.95×10^{-8} M, equivalent to the concentration of rimorphin on a molar basis, failed to antago-

Fig. 1. Effect of dynorphin on glucose induced insulin secretion from the isolated perfused pancreas. Insulin values represent the mean±s.e. of 8 rats (•—•) and shadow indicates glucose-induced insulin secretion without dynorphin (control). Asterisks (*) show significant difference from control values (p<0.05)
nize the inhibitory action rimorphin on glucose-induced insulin secretion (Table 1).

Fig. 2. Effect of rimorphin on glucose induced insulin secretion from the isolated perfused pancreas.
Insulin values represent the mean ± s.e. of 4 rats (●—●, at 3.95 × 10⁻⁸ M) rimorphin and of 8 rats (○—○, at 3.95 × 10⁻¹¹ M rimorphin). Shadow in this figure indicates glucose-induced insulin secretion without rimorphin (control).
Asterisks (*) show significant difference from control values (p < 0.05)

<table>
<thead>
<tr>
<th>Substance</th>
<th>Decrease in IIR (ng • min⁻¹ • ml⁻¹)</th>
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<tbody>
<tr>
<td>Rimorphin*</td>
<td>21.2 ± 6.3 (4)</td>
</tr>
<tr>
<td>Rimorphin* + phentolamine (10⁻⁶ M)</td>
<td>1.2 ± 3.9 (8)</td>
</tr>
<tr>
<td>Rimorphin* + naloxone</td>
<td>28.2 ± 19.7 (4)</td>
</tr>
<tr>
<td>Rimorphin* + naloxone (1.2 × 10⁻⁶ M)</td>
<td>1.8 ± 3.5 (4)</td>
</tr>
<tr>
<td>Dynorphin*</td>
<td>44.2 ± 12.3 (8)</td>
</tr>
<tr>
<td>Morphine*</td>
<td>77.9 ± 14.0 (3)</td>
</tr>
</tbody>
</table>

*3.95 × 10⁻⁸ M.
Values are given in terms of mean ± s.e. with experimental numbers in parentheses.
Effect of rimorphin on glucose-induced insulin secretion in the perfused pancreas of a cysteamine-treated rat

Oral administration of cysteamine (300 mg/kg) depleted somatostatin from the pancreatic islets; immunohistochemically few somatostatin-producing cells of the islets remained (data are not shown). In these animals rimorphin also inhibited glucose-induced insulin secretion from the perfused pancreas. The decrease in IIR produced by rimorphin in cysteamine-treated pancreas was $16.6 \pm 2.9 \text{ ng}\cdot\text{min}^{-1}\cdot\text{ml}^{-1}$. Thus, rimorphin appears to inhibit glucose-induced insulin secretion through other mechanisms than somatostatin.

Effect of propranolol and phentolamine on rimorphin action on glucose induced-insulin secretion from the perfused pancreas

Propranolol ($7.5 \times 10^{-6}$ M) was infused through a side arm tube to the perfused pancreas at 10 min after start of the infusion of glucose (150 mg/100 ml). The pancreas which had been perfused in advance with both glucose and propranolol for 20 min was perfused with rimorphin for the midst 10 min. Under these conditions propranolol failed to interfere with the inhibitory action of
rimorphin on glucose-induced insulin secretion. On the other hand, the inhibitory effect of rimorphin on glucose-induced insulin secretion was significantly attenuated by phentolamine (10^{-6} M), whereby the decrease in IIR was 1.2 ± 3.9 ng•min•ml^{-1} (Table 1).

**DISCUSSION**

Effects of opioid peptides on insulin secretion have been controversial, i.e., beta-endorphin (Ipp et al. 1978, 1982) and beta-lipotropin (Schwandt et al. 1981) stimulated insulin secretion, or enkephalins inhibited it (Kanter et al. 1980; Rudman et al. 1983). Recently Green et al. (1980, 1983) reported that dynorphin stimulated insulin secretion via an increase in both cyclic AMP and calcium influx in the islets. On the contrary, rimorphin and dynorphin as well as morphine (3.95 x 10^{-8} M) inhibited glucose-induced insulin secretion in our study. The discrepancies between the results of Dr. Green and his coworkers and ours may be ascribed to the methodological differences, i.e., in our study the perfusion technique of the isolated pancreas was used, whereas in his study the incubation of the isolated islets was done. We used dynorphin (1–17) whereas he used dynorphin (1–13). The concentration of dynorphin was higher in our study than that in theirs. We have reported already that met-enkephalin at high concentration (1.75 x 10^{-5} M) inhibited both glucose and acetylcholine-induced insulin secretion (Toyota et al. 1982). According to a report of Scala-Guenot and McIntosh (1985), a high concentration of met-enkephalin (10^{-5} M) stimulated somatostatin and insulin from the perfused rat pancreas in the presence of glucose (300 mg/100 ml). This result was inconsistent with our conclusion but, at least, showed that in this case somatostatin was not essential for paracrine regulation of insulin secretion. However, physiologic action of met-enkephalin in the concentration of 10^{-6} M was inhibitory on somatostatin and stimulatory on insulin. They concluded that enkephalinergic pathways were potentially involved in the paracrine regulation of insulin (Scala-Guenot and McIntosh 1985). In contrast to this the present data showed that even when somatostatin was depleted from the pancreatic islets by cysteamine hydrochloride, the inhibitory action of rimorphin on glucose-induced insulin secretion was not interrupted. This result suggests that it is unlikely that rimorphin acts on insulin by mediation of somatostatin as a paracrine mechanism.

The similarity of insulin responses to rimorphin and morphine suggests an essential role of endogenous morphine-like substances via opiate receptors. In a high concentration of naloxone which has a high affinity for mu-type opiate receptors, all receptor subtypes may be occupied. Therefore, it remains uncertain that in the present experiments rimorphin acts specifically on mu type of opiate receptor. At a low concentration (3.95 x 10^{-8} M), molarly equivalent to rimorphin, naloxone failed to interfere with rimorphin action. At least other mechanisms, besides a direct action on opiate receptor, may play a role in regulation of insulin secretion by rimorphin.
Met-enkephalin-Arg⁹-Gly⁷-Leu⁸ have been localized by immunohistochemistry to nerve fibers in the pancreas (Shimosegawa et al. 1983). Therefore, either sympathetic nerve fibers in the islets or catecholamines released from them may act cooperatively on opioid peptides. This assumption is supported by the result that phentolamine attenuated the morphine action on insulin. It remains yet to be solved that a characteristic feature of insulin secretion in NIDDM (non-insulin dependent diabetes) is ascribed to this phenomenon. Unless a possibility of hypersensitivity to enkephalins in NIDDM is disproved, low insulin response in NIDDM to glucose stimulus may be partly due to inhibitory action of opioid peptides on insulin.

References


