The Roles of Glucagon and Adrenal Epinephrine in Mediating Hyperglycemia Induced by Third Cerebroventricular Injection of Bombesin

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Abstract

The roles of glucagon and adrenal epinephrine in mediating bombesin-induced central hyperglycemia were further studied in anesthetized rats. Bombesin (10^-9 mol) injected into the third cerebral ventricle produced an increase in plasma concentrations of glucose, glucagon, and epinephrine. Prior bilateral adrenalectomy completely prevented the hyperglucagonemic and hyperglycemic responses to third cerebral ventricle injection of bombesin. These results support the view that bombesin-induced increases in plasma glucose and glucagon are fully dependent on adrenal epinephrine secretion. Furthermore, during constant intravenous infusion of somatostatin, the hyperglycemic response to third cerebral ventricle injection of bombesin was not significantly influenced despite complete inhibition of the increase in plasma glucagon. Therefore, it is suggested that bombesin-induced central hyperglycemia is mainly mediated by epinephrine itself rather than via epinephrine-stimulated glucagon secretion.

Bombesin, a tetradecapeptide, has been reported to produce a central nervous system (CNS)-mediated hyperglycemia associated with hyperglucagonemia and relative (or absolute) hypoinsulinemia (Brown et al., 1977; and 1979; Iguchi et al., 1984; Gunion et al., 1984). In addition, this bombesin-induced increase in plasma glucose and glucagon concentrations has been demonstrated to be fully dependent on adrenal epinephrine secretion (Brown et al., 1977 and 1979).

Since both glucagon and epinephrine can induce hyperglycemia, the effect of epinephrine may be mediated either by epinephrine itself or via stimulation of glucagon secretion. M. Brown et al. (1979) have suggested that endogenously secreted epinephrine after the injection of bombesin into the CNS produces hyperglycemia mainly via stimulation of glucagon secretion. On
the other hand, it has been reported that exogenously infused epinephrine can stimulate hepatic glucose output independent of any effect on insulin or glucagon secretion (Sacca et al., 1978; Rizza et al., 1979; Cherrington et al., 1984).

We recently reported that neostigmine injected into the third cerebral ventricle stimulated the secretion of epinephrine, which in turn resulted in producing hyperglycemia in the absence of a rise in the plasma glucagon concentration during constant intravenous infusion of somatostatin (Iguchi et al., 1986). Thus, the present study was aimed to further examine whether bombesin-induced central hyperglycemia was due to secreted epinephrine itself, or mediated via epinephrine-stimulated glucagon secretion.

Materials and Methods

Male albino Wister rats (Kearly Co., Nagoya, Japan), weighing 280–300 g, were used. The animals were kept in an air-conditioned, light-dark cycle laboratory, and permitted free access to food and water until they were anesthetized at 9:00–9:30 A.M. Following anesthesia with pentobarbital sodium (40 mg/Kg, ip), a Silastic® catheter (Dow Corning Co., Midland, MI) was introduced into one of the hepatic veins for repeated blood samplings by the transjugular hepatic vein cannulation technique (Iguchi et al., 1979). Each rat with the catheter in the hepatic vein was moved onto a stereotaxic apparatus. The method of stereotaxic microinjection of 1 μl of bombesin (10⁻⁹ mol/1 μl) into the third cerebral ventricle has been described previously (Iguchi et al., 1985). Bombesin was obtained from the Peptide Institute, Inc. (Osaka, Japan).

Bilateral adrenalectomy was performed 7 days before the experiment, and synthetic corticosteroid (100 μg/0.2 ml; betamethasone sodium phosphate, Rindelon®, Shionogi Pharmaceuticals, Japan) was administered subcutaneously on alternate days as described previously (Iguchi et al., 1986). The adrenalectomized rats had free access to laboratory chow and saline solution until the experiment was started. Microinjections of bombesin into the third cerebral ventricle were also given to the adrenalectomized rats.

Using another Silastic catheter introduced into the right femoral vein, constant intravenous infusion of somatostatin (1.0 μg·Kg⁻¹·min⁻¹; Peptide Institute Inc., Osaka, Japan) was started 30 minutes before injection of bombesin (10⁻⁹ mol/1 μl) into the third cerebral ventricle, and was continued throughout the study, as described previously (Iguchi et al., 1986). This somatostatin infusion was done in intact or adrenalectomized rats.

After the experiments, the animals were killed by the intravenous injection of an overdose of pentobarbital, and the position of the hepatic venous cannula was verified by inspection and palpation in each rat. The brains were then perfused with and fixed in 10% neutral formalin. After embedding, the brains were stained, and the injection site was examined in histological sections.

Samples of hepatic venous blood were taken immediately before and at various intervals after the injection of bombesin. Plasma glucose was determined enzymatically with a YSI 23A glucose autoanalyzer (Yellow Springs Instrument Co., Yellow Spring, OH). Venous plasma Immunoreactive insulin (IRI) and glucagon (IRG) were measured with commercially available radioimmunoassay kits (insulin, Insulin-Riabead®, Dainabot RI Laboratories, Tokyo, Japan; glucagon, Glucagon kit “Daiichi”®, Daiichi RI Institute, Tokyo, Japan). Venous plasma epinephrine and norepinephrine were measured by high-performance liquid chromatography with an electrochemical detector, as previously described in detail (Iguchi et al., 1986).

For statistical analysis Student’s t-test was used. Differences were considered significant when P<0.05.

Results

Fig. 1 illustrates the changes in hepatic venous plasma glucose, IRI, IRG, epinephrine, and norepinephrine concentrations after injection of bombesin (10⁻⁹ mol) into the third cerebral ventricle. The plasma glucose concentration increased significantly 30 min after the injection and
Fig. 1. Time-related changes in hepatic plasma glucose, immunoreactive insulin (IRI), immunoreactive glucagon (IRG), epinephrine and norepinephrine concentrations after microinjection of bombesin (10^{-9} mol, 1 \mu l; \bullet-\bullet) or saline (1 \mu l; \bigcirc-\bigcirc) into the third cerebral ventricle. Values are mean±SE for 6–8 rats. *P<0.05, significant differences compared with saline-treated controls at identical times. The baseline (preinjection) hepatic venous plasma glucose, IRI, IRG, epinephrine, norepinephrine concentrations were:

<table>
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<tr>
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<th>Saline</th>
<th>Bombesin</th>
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<tr>
<td>glucose (mg/100 ml)</td>
<td>138±4</td>
<td>139±5</td>
</tr>
<tr>
<td>IRI (\mu U/ml)</td>
<td>28±2</td>
<td>30±3</td>
</tr>
<tr>
<td>IRG (pg/ml)</td>
<td>48±8</td>
<td>45±5</td>
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<tr>
<td>epinephrine (ng/ml)</td>
<td>0.17±0.05</td>
<td>0.11±0.01</td>
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<tr>
<td>norepinephrine (ng/ml)</td>
<td>0.39±0.08</td>
<td>0.34±0.02</td>
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continued to increase until 120 min. The IRG concentration reached a peak value 30 min after the injection and then decreased gradually. The epinephrine concentration increased significantly 30 min after the injection and maintained significantly high values until 120 min. No significant differences in plasma IRI or norepinephrine concentrations were observed between the rats injected with bombesin and those injected with saline.

Hepatic venous plasma concentrations of glucose, IRG, and IRI after the bombesin (10^{-9} mol) injection into the third cerebral ventricle remained stable throughout the series of experiments in the bilateral adrenalectomized rats. There were no significant differences in plasma glucose, IRG, and IRI concentrations between the adrenalectomized rats injected with bombesin and those injected with saline. Thus, bombesin-induced increases in plasma IRG and glucose concentrations were completely abolished by prior bilateral adrenalectomy. These results are summarized in Fig. 2.

Fig. 3 shows the effects of somatostatin infusion on glucose and IRG responses to bombesin. During constant intravenous infusion of somatostatin, the hepatic venous plasma glucose concentrations significantly
increased at 30 min and kept increasing until 120 min after bombesin (10^{-9} \text{ mol}) injection into the third cerebral ventricle. The values for plasma glucose concentrations at sampling times only slightly reduced, but were not significantly different from the corresponding values in the rats given bombesin but not somatostatin (compare Fig. 3 with Fig. 1). In this situation the bombesin-induced elevation in the IRG concentration was totally suppressed. No significant changes in hepatic venous plasma IRG concentrations were found after third cerebral ventricle injection of bombesin in comparison with saline-treated controls. Furthermore, in bilateral adrenalectomized rats with somatostatin infusion, bombesin-induced hyperglycemia was not observed.

Fig. 2. Effects of prior bilateral adrenalectomy on hepatic venous plasma glucose, immunoreactive glucagon (IRG), and immunoreactive insulin (IRI) concentrations after third cerebral ventricle injection of bombesin (10^{-9} \text{ mol}, 1 \mu l; closed bar) or saline (1 \mu l; open bar). Bombesin was also administered into the same site in sham-operated rats (hatched bar). Values are expressed as the calculated area under the curves over the interval 0 to 120 minutes. Each bar is mean±SE for 5-7 rats. *P<0.05 (statistical significance).
Discussion

Bombesin is well known as a neuromodulator producing hyperglycemia through the stimulation of the CNS in rats (Brown et al., 1977 and 1979; Iguchi et al., 1984; Gunion et al., 1984) and dogs (Brown 1983). In 1977, M. Brown et al. reported that bombesin injected into the cisternum magnum of rats produced hyperglycemia in doses which were much smaller than those required with intravenous injection. Thereafter, bombesin-induced central hyperglycemia has also been observed in rats after injecting bombesin into the lateral cerebral ventricle (Brown et al., 1979), or into the hypothalamus (Iguchi et al., 1984). Furthermore, this hyperglycemia has been shown to be accompanied by hyperglucagonemia and relative (or absolute) hypoinsulinemia (Brown et al., 1977 and 1979; Iguchi et al., 1984). Consistent with these reports, the present study also indicates that bombesin injected into the third cerebral ventricle produces hyperglycemia with hyperglucagonemia and relative hypoinsulinemia.

M. Brown et al. (1979) have demonstrated that bombesin administered into the CNS causes an increase in plasma epinephrine but not plasma corticosterone. They (Brown et al. 1977 and 1979) have also reported that prior adrenalectomy but not hypophysectomy completely prevents hyperglycemia and hyperglucagonemia after intracisternal injection of bombesin. In the present study, bombesin injected into the third cerebral ventricle stimulated the secretion of epinephrine, and prior bilateral adrenalectomy completely abolished bombesin-induced hyperglucagonemia and hyperglycemia. These results are in agreement with the above findings of M. Brown et al. and support the view that bombesin-induced increases in plasma glucose and glucagon are fully dependent on adrenal epinephrine secretion.

Thus, the increase in plasma glucose and glucagon concentrations after third cerebral ventricle injection of bombesin is secondary
to adrenal epinephrine secretion. However, two possible mechanisms by which endogenously secreted epinephrine after bombesin results in producing hyperglycemia are postulated. First, increased epinephrine itself results in development of hyperglycemia independent of glucagon secretion (Sacca et al., 1978; Rizza et al., 1979; Cherrington et al., 1984). Second, the rise in plasma epinephrine produces hyperglycemia by virtue of its action in increasing plasma glucagon (Ezdinli and Sokal 1966; Gerich et al., 1976; Chideckel et al., 1977). In the present study, during constant intravenous infusion of somatostatin, the increase in plasma glucagon after third cerebral ventricle injection of bombesin was completely inhibited, whereas the hyperglycemic response to bombesin was not significantly influenced. If bombesin-induced central hyperglycemia is mediated by epinephrine-induced glucagon secretion, somatostatin should prevent this hyperglycemia (Weir et al., 1974; Gerich et al., 1976). Our observation therefore suggests that bombesin injected into the third cerebral ventricle can cause hyperglycemia independent of glucagon secretion. Although it has not yet been thoroughly clarified whether or not somatostatin itself directly affects the rates of either glucose uptake or glucose production (Cherrington et al., 1977; Sacca et al., 1979), our previous report (Iguchi et al., 1986) demonstrated that the hyperglycemic response to endogenously secreted epinephrine is not influenced by this constant somatostatin infusion. Furthermore, bombesin-induced hyperglycemia was not observed in bilateral adrenalectomized rats with constant somatostatin infusion, supporting the theory that the hyperglycemic response to bombesin is dependent on the secretion of epinephrine. Thus, it is suggested that endogenously secreted epinephrine induced hyperglycemia after third cerebral ventricle injection of bombesin is mediated by epinephrine itself rather than via its stimulatory effect on glucagon secretion. However, in this in vivo study, despite no significant effect of somatostatin infusion on bombesin-induced hyperglycemia, we cannot completely exclude the possibility that the hyperglycemic effect induced by third cerebral ventricle injection of bombesin may, in very small part, be the result of the increased plasma glucagon concentration.

On the other hand, M. Brown et al. (1979) have suggested that epinephrine-induced hyperglucagonemia is mainly responsible for bombesin-induced central hyperglycemia because systemic bolus administration of somatostatin abolished hyperglucagonemic and hyperglycemic responses to the intracisternal injection of bombesin. Although the reason for the difference between their results and ours in the present study is not clear, it appears unlikely that the different injection sites of bombesin (third cerebral ventricle vs. cisternum magnum) and the different methods of anesthesia (pentobarbital vs. ether) may be responsible for the different results, because the two studies have similar effects on the secretion of epinephrine and epinephrine-induced glucagon. However, it is not unreasonable to propose that the different methods of somatostatin administration (constant infusion vs. bolus injection) may explain the different results in the two studies. Systemic bolus injection of somatostatin with a large dose might interfere with the effect of epinephrine independent of glucagon availability (Sacca et al., 1979). Moreover, the regimen of somatostatin administration might inhibit the hyperglycemic effect of bombesin within the CNS, because the hyperglycemic response to bombesin injected centrally has been reported to be suppressed by the central administration of somatostatin (Brown et al., 1980 and 1983). We think that the constant intravenous infusion method is more effective, because the duration of the action of a
single dose of somatostatin is brief.

In conclusion, the present studies indicate that bombesin injected into the third cerebral ventricle stimulates the secretion of epinephrine, which in turn results in producing hyperglycemia independent of epinephrine-stimulated glucagon secretion in anesthetized rats.

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References


