Effects of corticotropin-releasing hormone on insulin and glucagon secretion in mice

Sven Karlsson¹ and Bo Ahrén¹,²

Departments of Pharmacology¹ and Surgery², Lund University, Lund, Sweden

Abstract. Besides in the brain, corticotropin-releasing hormone occurs in the pancreas. Therefore, its effects on plasma levels of insulin and glucagon were investigated in vivo in the mouse. At 2 min after CRH injection (0.5–8.0 nmol/kg), plasma insulin was lowered: by 4.0 nmol/kg from 38 ± 4 to 28 ± 2 mU/l (P < 0.05). Plasma insulin was lowered also at 6 min, whereas at 10 min, plasma insulin levels were elevated (P < 0.05). Plasma glucagon levels were slightly lowered (P < 0.05) at 10 min after CRH injection, whereas plasma glucose was slightly elevated (P < 0.05) at 6 min after injection but not at 2 or 10 min. The effects of CRH on the plasma insulin and glucagon response to iv injections of half-maximal dose levels of glucose (2.8 mmol/kg) or the cholinergic agonist carbachol (0.16 μmol/kg) were also investigated. CRH, 4.0 nmol/kg, however, could not influence the plasma insulin or glucagon levels after the iv injection of either glucose or carbachol. Thus, CRH slightly affects basal plasma levels of insulin and glucagon in mice. In contrast, stimulated insulin and glucagon secretions are not affected by CRH. Peripheral CRH may therefore be of slight importance for the regulation of basal plasma levels of insulin and glucagon in the mouse.

Corticotropin-releasing hormone is a peptide consisting of 41 amino acids that has been demonstrated in the brain (Vale et al. 1983; Tilders & Berkenbosch 1986). From its hypothalamic site of occurrence, the peptide is thought to be released into the pituitary portal circulation as a response to various forms of stress and thereby activate the secretion of ACTH (Rivier et al. 1982; Plotsky & Vale 1984; Tilders et al. 1985). Intracerebral CRH has also been suggested to be of physiologic-
the effects of CRH on the release of PP have consistently shown that CRH is able to stimulate PP secretion both in man (Lytras et al. 1984) and in dogs (Konturek et al. 1985). In contrast, reports on the influences of CRH on insulin secretion have presented conflicting results: CRH has been shown to inhibit insulin secretion from the perfused rat pancreas (Moltz & Fawcett 1985a), but to enhance plasma insulin levels in vivo in the rat (Torres-Aleman et al. 1984), and to be without effect on plasma insulin levels in man (Lytras et al. 1984). The possible influences of CRH on glucagon secretion have not been studied in great detail; in isolated rat islets, CRH has been shown to stimulate glucagon secretion (Moltz & Fawcett 1985b), but in man CRH did not affect plasma glucagon levels (Lytras et al. 1984).

Since the reports on the influence of CRH on insulin secretion have been conflicting and since the effects of CRH on glucagon secretion are not clearly established, we performed the present study in the mouse and investigated the effects of synthetic CRH on plasma levels of insulin, glucagon and glucose in the basal state and after stimulation with glucose and the cholinergic agonist carbachol.

Materials and Methods

Animals

Female mice of the NMRI strain (Anticimex, Stockholm, Sweden), weighing 25–30 g, were used. The animals were fed a standard pellet diet (Astra-Ewos, Södertälje, Sweden), and tap water.

Experimental procedure

Synthetic rat CRH (Bacchem Inc, Torrance, CA), dissolved in saline-0.1% gelatin, was injected iv into unanaesthetized mice at dose levels in the range of 0.5 to 8.0 nmol/kg. Controls were injected with saline-gelatin. Blood samples were taken by the orbital puncture technique at 2, 6, or 10 min after the injection. In the second series of experiments, unanaesthetized mice were injected iv with CRH (4.0 nmol/kg) alone or together with D-glucose (2.8 mmol/kg) or carbachol (0.16 µmol/kg) (British Drug Houses Ltd, Poole, England); controls were injected with saline-gelatin or glucose or carbachol alone. Blood samples were taken at 2 min after the iv injection. At this time point, the peak levels of the increased plasma insulin and glucagon levels after iv injection of glucose or carbachol are seen (Ahrén & Lundquist 1981, 1986).

Determinations of insulin, glucagon and glucose

Plasma levels of immunoreactive insulin and glucagon were determined radioimmunochemically (Heding 1966; Ahrén & Lundquist 1982a). The antiserum used for the determination of plasma glucagon is specific for pancreatic glucagon (Milab, Malmö, Sweden). Plasma glucagon levels were determined with the glucose oxidase method (Bruss & Black 1978).

Statistics

The results are expressed as mean ± SEM. Student's t-test was used to test the degree of significance.

Results

Effects of CRH on basal plasma levels of insulin, glucagon, and glucose

CRH, injected at dose levels ranging from 0.5 to 8.0 nmol/kg iv to mice, did slightly reduce basal plasma levels of insulin (P < 0.05 when com-
Comparing pooled observations with CRH with that in controls). There were, however, no clear dose-response relationship for the effect of CRH on plasma insulin levels. In contrast, no significant effect of CRH on basal plasma glucagon levels in samples taken at 2 min after the injection was seen. No significant influences on basal plasma glucose levels were observed at 2 min after injection of CRH (Fig. 1).

Fig. 2 shows the plasma levels of insulin, glucagon and glucose at 2, 6, and 10 min after injection of CRH at a concentration of 4.0 nmol/kg. It is seen that compared with controls, plasma insulin levels were lowered at 2 and 6 min by CRH: at 2 min, they were 38 ± 4 mU/l in controls and 28 ± 2 mU/l in animals given CRH (P < 0.05), and at 6 min after injection 54 ± 5 mU/l in controls against 37 ± 5 mU/l in the CRH group (P < 0.05). At 10 min after injection, plasma insulin levels were higher in CRH-injected animals than in controls (P < 0.05). In this series of experiments, plasma glucagon levels were increased at 2 min after injection of CRH: to 173 ± 20 ng/l against 103 ± 16 ng/l in controls (P < 0.05). At 6 min after injection, however, no significant difference was observed between the groups with regard to plasma glucagon levels, whereas at 10 min after CRH injection, plasma glucagon levels had decreased when compared with controls (P < 0.05). Plasma glucose levels were slightly elevated at 6 min after CRH injection: to 10.8 ± 0.3 mmol/l against 9.7 ± 0.3 mmol/l in controls (P < 0.05), whereas at 2 and 10 min after CRH injection plasma glucose levels were not different from those in the controls.

**Fig. 2.**
Plasma levels of insulin (upper panel), glucagon (middle panel), and glucose (lower panel) at 2, 6, or 10 min after iv injection of CRH (4.0 nmol/kg) or saline-gelatin (control). There were 20 animals in each group. Means ± SEM are shown. P indicates probability level of random difference between groups.

□ Control. ■ CRH.
Effects of CRH on plasma levels of insulin, glucagon and glucose after injection of glucose or carbachol

Fig. 3 shows that the iv injection of glucose (2.8 mmol/kg) or the cholinergic agonist carbachol (0.16 µmol/kg) markedly increased plasma levels of insulin. CRH (4.0 nmol/kg) could not, however, affect this insulin response to glucose or carbachol. Furthermore, carbachol increased plasma glucagon levels markedly, but, again, CRH (4.0 nmol/kg) could not affect this response.

Discussion

Plasma levels of CRH have been shown to be low or undetectable (Stalla et al. 1986; Cunnah et al. 1987). It could therefore be anticipated that CRH functions not as a circulating hormone but as a local regulator within the tissue where it is produced. Since CRH-immunoreactivity has been demonstrated in endocrine cells in the pancreas (Petrusz et al. 1983) and in intrapancreatic nerves (Moltz et al. 1985), CRH might be suggested to be a local regulator of islet function, as has been postulated for other intrapancreatic neuropeptides (Ahrén et al. 1986). While previous studies thus have shown that CRH stimulates the secretion of PP (Lytras et al. 1984; Konturek et al. 1985), results of studies on the influences of CRH on insulin and glucagon secretion are not consistent.

Earlier studies have shown that CRH in vivo either has no effect on basal plasma insulin and glucagon levels in man (Lytras et al. 1984) or enhances basal plasma insulin levels in rats (Torres-Aleman et al. 1984). We show here that CRH reduces basal plasma insulin levels during the first 6 min after its injection into mice. Our results may be at variance with those reported in the earlier study in rats, where an increase in plasma insulin levels was observed (Torres-Aleman et al. 1984). However, in that study, a preceding lowering of plasma insulin levels may have been undetected and the authors may have noted the late elevation of basal plasma insulin levels only. The plasma half-life of CRH after iv injection is short, in man approximately 9 min (Stalla et al. 1986). Therefore, it is not unlikely that a direct effect of CRH vanishes after the initial 6 min. However, it can not be excluded that true species differences exist with regard to effects of CRH on insulin secretion. On the other hand, it has been shown that CRH inhibits insulin secretion from the isolated rat pancreas (Moltz & Fawcett 1985a). It might therefore be suggested that the direct effect of CRH is to reduce plasma insulin levels. Following the initial lowering of plasma insulin levels, a rebound stimulation is seen, presumably due to the transient hyperglycaemia.

The exact site of effects of CRH on insulin secretion is not obvious, since basal plasma insulin levels depend on both secretion and elimination of insulin. It is not unlikely, though, that the changes in plasma insulin levels after CRH injection reflect alterations in insulin secretion, since a direct inhibition of insulin secretion followed by a stimulatory off-response was observed upon administration of CRH to the perfused rat pancreas (Moltz & Fawcett 1985a). CRH seems, however, to lack effects on insulin secretion from isolated islets (Moltz & Fawcett 1985b). It might therefore be proposed that CRH acts indirectly to affect basal insulin secretion, a suggestion corroborated by our lack of a clear relationship between dose of CRH and lowering of plasma insulin levels.

Basal plasma glucagon levels were not consistently altered by CRH at 2 min after injection. In a previous study in rat islets it was demonstrated that CRH is capable of stimulating glucagon secretion (Moltz & Fawcett 1985b). In some series of experiments we observed enhancement of plasma glucagon levels after injection of CRH. However, the degree of significance was low (Fig. 2), which probably explains why this effect was not visible in other series (Fig. 3). In combination with the lowered basal plasma insulin levels, the slight hyperglucagonaemia could result in hyperglycaemia, which, though slight, was regularly observed at 6 min after injection and in some animals also at 2 min after injection. This pattern of alterations, hypoinsulinaemia, hyperglucagonaemia and hyperglycaemia, is commonly seen during stress (Ahrén et al. 1986) and it might therefore be suggested that CRH is of some importance for the regulation of basal levels of insulin and glucagon during stress. However, the low potency of the effects of CRH suggests that this peptide is of no great importance. It is of interest that the effects of CRH demonstrated in the present study after peripheral injection of the peptide resemble those found after intracerebroventricular administration (Brown et al. 1982).
This might imply that CRH induces hyperglycaemia, hyperglucagonaemia, and hypoinsulinemia both through central and peripheral actions.

CRH did not affect the insulin or glucagon responses to iv injections of glucose and the cholinergic agonist carbachol. Since the doses used for glucose and carbachol are not maximal doses (Ahrén & Lundquist 1981, 1982b), and since the increase in plasma levels of insulin and glucagon at 2 min after injection of glucose and carbachol reflect stimulation of insulin and glucagon secretion, our results suggest that CRH does not directly affect stimulated insulin and glucagon secretion. This restricts a possible importance of CRH in the regulation of basal islet hormone secretion.

In summary, our present study on the in vivo influences of CRH has shown 1) that CRH transiently lowers basal plasma insulin levels, induces a slight hyperglycaemia, and causes a variable enhancement of plasma glucagon levels, and 2) that CRH does not affect glucose- or carbachol-induced insulin secretion or carbachol-induced glucagon secretion. Our study thus suggests that CRH is of no great importance for the regulation of islet hormone secretion in vivo in the mouse, though a slight contribution to the regulation of basal islet hormone secretion cannot be excluded.

Acknowledgments

The technical assistance of Lena Kivist is gratefully acknowledged. This study was supported by the Swedish Medical Research Council (14X-6834), by Nordisk Insulinfond, Gentofte, Denmark, by the Swedish Diabetes Association, Stockholm, Sweden, by Påhlssonfonden, Malmö, Sweden, Jaenssson's Stiftelse, Stockholm, Sweden, Carboofordska Stiftelsen, Lund, Sweden, and by the Medical Faculty, Lund University, Lund, Sweden.

References


Ahrén B & Lundquist I (1982b): Interaction of vaso-

active intestinal peptide (VIP) with cholinergic stimulation of glucagon secretion. Experientia 38: 405–406.


Received July 30th, 1987.
Accepted October 19th, 1987.

Dr Sven Karlsson,
Department of Pharmacology,
Sölvegatan 10,
S-22362 Lund,
Sweden.