Gastrointestinal hormones are known to influence the secretion of insulin. The more marked insulin release induced by an oral intake of glucose compared to that obtained by i.v. administration of the same amount of glucose has been largely attributed to these hormones (see recent review). It has long been suggested that cholecystokinin (CCK) played a role in this gastrointestinal potentiation of insulin release. CCK is released by intake of a meal and administration of CCK has been shown to stimulate insulin release in several species. However, results showing no effect of CCK in this respect have also been published, suggesting that the insulin secretory effect demonstrated was caused mainly by impurities.

CCK has been shown to occur in several different forms, and CCK variants with 39, 33, and 8 amino acids have been described. The physiological significance of these different CCK variants is still far from having been elucidated although most of the evidence available suggests that the biological activity of CCK is largely confined to the C-terminal octapeptide (CCK-8).

In the present study the in vivo effects of two different CCK molecules on basal and stimulated insulin release were studied in the mouse. The two forms of CCK used were a pure preparation of CCK-39 and the carboxylterminal octapeptide, CCK-8. The effects of these two peptides on basal insulin secretion as well as insulin release stimulated by D-glucose, the cholinergic agonist carbachol, and the β-adrenergic agonist L-isopropyl noradrenaline (L-IPNA) were compared. Additionally, influences of the muscarinic blocker methylatropine and the β-adrenergic blocker L-propranolol on CCK-induced insulin secretion were investigated.

Key-words: CCK-8; CCK-39; Cholecystokinin; Insulin secretion; Mouse.
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MATERIALS AND METHODS

Animals - Female mice of the NMRI strain (Laboratory Animal Breeding, Laven, Denmark), weighing 25-35 g, were used throughout the experiments. The animals were kept on a standard pellet diet (Astra-Ewos, Södertälje, Sweden), and tap water ad libitum before and during the experiments.

Drugs - Pure CCK-39 was kindly donated by Professor V. Mutt, Karolinska Institutet, Stockholm, Sweden. Synthetic CCK-8 (Kinevac®) was from Squibb, New Brunswick/N.J., U.S.A. Both peptides were dissolved in 0.9% NaCl with addition of 0.1% gelatine to avoid adsorption to tubes. D-glucose and carbachol were from British Drug Houses Ltd., Poole, England. L-isopropylnoradrenaline (L-IPNA) was from Sterling-Winthrop Research Institute, Rensselaer/N.Y., U.S.A.; L-propranolol from ICI, Macclesfield, England; methylatropine from Vitrum AB, Stockholm, Sweden. All these agents were dissolved in 0.9% NaCl.

Effects of CCK on basal insulin secretion - The peptides were administered i.v. into a tail vein. Ten μl/g body weight were injected rapidly. Several different doses were given. Two min after injection, 250 μl blood were drawn by orbital puncture using commercial constriction pipettes. The blood sampling did not last more than 5 sec. The mice were unanesthetized all the time. In preliminary experiments it had been found that the peak level of immunoreactive insulin (IRI) following an injection of CCK-39 and CCK-8 was recorded about 2 min after the injection.

Effects of methylatropine and L-propranolol on CCK-induced insulin release - Methylatropine (8.2 μmol/kg body weight), L-propranolol (9.6 μmol/kg body weight) or saline, respectively, were injected i.p.; 10 μl/g body weight were given. Twenty min later, CCK-39 (2.12 nmol/kg body weight), or CCK-8 (3.18 nmol/kg body weight) or saline, respectively, were given i.v.; 10 μl/g body weight were given. Control groups injected i.v. with carbachol (0.16 μmol/kg body weight) or L-IPNA (0.34 μmol/kg body weight) were included. Two min after the i.v. injection, blood was collected in all groups, except the L-IPNA-injected group, where the blood was sampled after 5.5 min.

Effects of CCK on glucose-, carbachol-, and L-IPNA-stimulated insulin release - Each of the two peptides was injected i.v. into a tail vein, 0.53 nmol/kg body weight was given; the volume load was 5 μl/g body weight. The dose chosen was a threshold dose without effect on basal insulin secretion. Fifteen sec after peptide injection, a rapid i.v. injection of a half-maximal dose of D-glucose, carbachol, or L-IPNA, respectively, was given. The volume load was, again, 5 μl/g body weight. This sequence of injection was chosen in order to mimic the physiological changes following a meal, where gut and pancreatic peptides may reach the insulin cells before the nutrients.23 Two min (D-glucose and carbachol) or 5.5 min (L-IPNA), respectively, after the last injection, blood was drawn by the orbital puncture technique. Repeated experiments in this laboratory had shown that maximal concentration of IRI in mouse plasma following a rapid i.v. injection of D-glucose or carbachol is reached after about 2 min, and following injection of L-IPNA after about 5.5 min. The doses used were 2.78 mmol/kg for D-glucose, 0.16 μmol/kg for carbachol, and 0.34 μmol/kg for L-IPNA, respectively.