Effects of cholecystokinin octapeptide on thermoregulatory responses and hypothalamic neuronal activity in the rat

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Summary. 1. Rats were chronically implanted with a hypothalamic cannula to allow chemical stimulation of the hypothalamus on the conscious animals in repeated experiments. Direct administration of cholecystokinin octapeptide (CCK-8) (20–60 ng) into the preoptic anterior hypothalamic area caused a dose-related fall in rectal temperature at ambient temperatures of 8°C and 22°C.

2. The hypothermia induced by CCK-8 was produced by a decrease in metabolism at an ambient temperature of 8°C, whereas at 22°C, it was caused by both a decrease in metabolism and an increase in cutaneous temperature.

3. However, at an ambient temperature of 30°C, intrahypothalamic administration of CCK-8 caused an insignificant change in thermoregulatory responses. Furthermore, neither intrahypothalamic injection of 0.9% saline nor intraperitoneal injection of CCK-8 (60 ng) had any effect on thermoregulatory responses at the ambient temperatures of 8°–30°C studied.

4. Under urethane anaesthesia, 59 single neurons in the preoptic anterior hypothalamic area were examined in 29 rats. Each animal was subjected to scrotal warming or cooling and to the administration of CCK-8. Microiontophoretic application of CCK-8 resulted in inhibition of the majority (75%) of cold-responsive neurons as well as excitation of the majority (77.8%) of warm-responsive neurons recorded in the preoptic anterior hypothalamic area. However, the majority (69%) of thermally unresponsive cells were not affected by CCK-8 application.

5. The data indicate that CCK-8, when administered intrahypothalaminically, excites warm-responsive neurons and inhibits cold-responsive neurons within the preoptic anterior hypothalamic area to induce hypothermia by promoting an increase in heat loss and a decrease in heat production.

Key words: Cholecystokinin - Thermoregulation - Hypothalamus - Neuronal activity - Metabolism - Vasodilation - Hypothermia

Introduction

There is much evidence to suggest that cholecystokinin (CCK)-related peptides have neuroregulatory roles in the central nervous system in addition to their well-known hormonal functions in controlling digestion (Dockray 1982).

For example, cholecystokinin octapeptide (CCK-8) administered into the cerebral ventricles had a potent satiety effect (Della-Fera and Bailey 1979). Intraventricular administration of CCK-8 was also shown to induce hypothermia in several species of animals (Katsuura and Itoh 1981; Morley et al. 1981; Zetler 1982).

The present investigation was an attempt to assess further the effects of administering CCK-8 into the preoptic anterior hypothalamic area of rat brain on such thermoregulatory responses as metabolic, respiratory and vasomotor activity and on hypothalamic neuronal activity, in order to test the possibility that CCK-8 plays a neuroregulatory role in body temperature regulation.

Methods

1. Experimental animals. Adult male Sprague-Dawley rats weighing 250–300 g were used in all experiments. Before use, the animals were housed individually in wire-mesh cages in a room maintained at 22 ± 2.0°C, with natural light-dark cycles. There was free access to tap water and granular chicken feed supplied by Taiwan Sugar Corporation. The thermoregulatory experiments were performed on restrained animals with implanted hypothalamic cannulae. Prior to the study, the animals were accustomed to being restrained for several hours at a time, with freedom of movement of their limbs and neck and they were conscious. The electrophysiological experiments, however, were performed on animals under urethane anaesthesia.

2. Cannula implantation. Drugs were administered into the preoptic anterior hypothalamic area, by means of stainless-steel cannulae consisting of a guide tube (0.81 mm outside diameter) with a snugly fitting trocar, and a cannula insert which could be introduced into the tube at the time of injection. The animals were anaesthetized with pentobarbital sodium (6 mg/100 g, i.p.) and placed in a Kopf stereotaxic apparatus. The cannula guide tubes with trocars were implanted into the preoptic anterior hypothalamic area using the stereotaxic atlas and co-ordinates of König and Klippel (1963). A period of 2 weeks was allowed for the animals to recover from operation.

3. Drug solutions. All drug solutions were prepared in pyrogen free glassware, which was heated for 5 h at 180°C before use. A 0.5-μl aliquot containing 0.9% saline or synthetic cholecystokinin octapeptide (CCK-8) (20–60 ng in 0.9% saline, pH 7.0, Boehringer-Mannheim, FRG) was
administered into the preoptic anterior hypothalamic area during 30 s.

4. Measurements of thermoregulatory response. The effects of the intrahypothalamic administration of 0.9% saline or CCK-8 on metabolic, respiratory and vasomotor activity and on the body temperature of conscious rats were assessed by using a partitional calorimeter for small animals (Lin et al. 1982a, 1983). The metabolic rate was calculated from the oxygen consumption of the animal and expressed in watts, assuming an RO = 0.83 so that 1 l of oxygen consumed per hour was equivalent to a heat production of 5.6 W.

The respiratory evaporative heat loss was calculated by measuring the increase in water vapour content in the helmet effluent air over that of the ambient air. The amount of water evaporated by the animal was calculated according to the equation: water loss = (water content of circuit air − water content of ambient air)× airflow. The evaporative heat loss, expressed as watts, was calculated from the evaporative water loss, assuming the latent heat of the vaporization of water to be 0.7 W · h⁻¹ · g⁻¹.

Rectal, foot skin and tail skin temperatures were measured using copper-constantan thermocouples. Rectal temperature was measured with a copper-constantan thermocouple enclosed in polyethylene tubing sealed at one end, inserted 60 mm into the rectum. All measurements were taken once per minute throughout the experiments, each variable being measured as a DC potential on a Hewlett-Packard 9871. Each minute, all temperatures, the metabolic rate and respiratory evaporative heat loss were calculated instantaneously by the computer and relayed back to the laboratory where they were displayed using an on-line printer Hewlett-Packard 9871.

Animals were exposed to each of the ambient temperatures for a period of 90 min to attain thermal balance before the drug injections were made. The maximal changes in rectal temperature, foot skin temperature, tail skin temperature, metabolic rate and respiratory evaporative heat loss produced within 60 min after the drug injections were collected at ambient temperatures of 8, 22 and 30°C. Each animal with implanted hypothalamic cannulae received several injections at an interval of 5–7 days to ensure recovery to the baseline and no remaining effects. After completion of the thermoregulatory experiments, the animals were killed with an overdose of pentobarbital sodium and perfused with 10% formalin solution. Later, sections of the fixed brains were cut and stained with thionin to verify the stereotaxic co-ordinates of the cannulae.

5. Electrophysiological recording. For the recording of unit activity, each animal was anaesthetized with urethane (1.25 g/kg, i.p.). The rectal temperature was maintained between 36.5° and 37.2°C by means of a water-prefused pad under the animal. All the fur of scrotal skin was removed. The animals were placed in the stereotaxic apparatus and the head fixed according to the König and Klippel co-ordinate system (1963). A piece of bone was removed from the right half of the skull and the underlying dura was removed. Recording of single unit discharges was made from the right half of the preoptic anterior hypothalamic area. The tips of the three-barreled micropipettes were broken back to produce a diameter of 4.1 ± 1.2 μm. The central barrel was filled with a solution of 4 mol/l NaCl, saturated with fast green dye, and used for extracellular recording. One of the side barrels was filled with a solution of 165 mmol/l NaCl at pH 7.8 in order to eliminate any possible current or pH artefacts, and to provide a current return path while ejecting CCK-8. The remaining side barrel was filled with synthetic CCK-8 (4.0 mmol/l in 165 mmol/l NaCl, pH 7.8, ejected as an anion). The iontophoretic unit contained a current balancing circuit. Conventional microiontophoretic techniques were used to study the effect of synthetic CCK-8 on the spontaneous firing of preoptic anterior hypothalamic neurons. The micropipette was lowered to the desired location in the hypothalamus, and a hydraulic microdrive used to slowly advance the micropipette. Single unit activity was processed using a standard cathode follower and amplification circuitry for extracellular spike potentials (Lin and Simon 1982). Impulses were counted at 1-s intervals by a WPI Scope Raster/Slipper Model 140 and displayed on a Grass Polygraph. The rectal temperature and scrotal temperature were displayed on the same polygraph record. The method used for thermal stimulation of the scrotum was similar to that described by Hellon and Misra (1973). The skin temperature was measured by a thermocouple cemented to the surface of the thermode which was in contact with the skin.

Thermal responsiveness was assessed by observing the changes in the neuronal discharge rate in response to warming and cooling of the scrotum. The neurons encountered were classified as thermally unresponsive, warm-responsive or cold-responsive. The warm-responsive neurons were excited by an increase in scrotal temperature; the cold-responsive neurons were excited by a decrease in scrotal temperature. Neurons that were not affected by changes in scrotal temperature were regarded as thermally unresponsive. A change of 25% or more in the spontaneous firing rate in response to application of CCK-8 was the criterion used to define whether a neuron was excited or inhibited. Neurons that were inhibited by temperature changes were not observed. Statistical significance of the tabulated data was determined using a χ² test and a 2 × 3 contingency table. Differences were considered statistically significant at P < 0.05. At the end of each experiment, the vertical location of the micropipette was recorded and 25 μA of negative current was passed through the micropipette for 10 min to deposit fast green dye at the site (Hosford and Haigler 1980). The locations of the fast green dye spots were used to verify the locations of the recording sites.

Results

1. Effects of intrahypothalamic administration of CCK-8 on thermoregulation

Rats with implanted unilateral hypothalamic cannulae were equilibrated in a partitional calorimeter for a period of about 90 min at the selected ambient temperature before drug injection. Direct administration of CCK-8 (20–60 ng in 0.5 μl, 0.9% saline) into the preoptic anterior hypothalamic area caused a dose-dependent fall in rectal temperature in conscious rats at ambient temperatures of 8° and 22°C (Table 1). For example, as depicted in Fig.1, the rectal temperature started to fall about 10 min after an intrahypothalamic dose of 40 ng of CCK-8, reached its minimal level at 60 min and returned to its original level at 100 min. The hypothermia induced by CCK-8 was brought