The Stimulatory Effect of Cholecystokinin Implanted in the Medial Preoptic Area on Luteinizing Hormone Secretion in the Ovariectomized Estrogen-Primed Rat

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Abstract

The effect of implantation of cholecystokinin(CCK)-8 into the medial preoptic area (MPO) through the chronically implanted guide cannula on the release of LH was examined in the ovariectomized estradiol-primed rat. An inner cannula with CCK-8 at the tip, as well as an empty inner cannula as the control, was implanted into the MPO at 12.00 h. Blood samples were obtained at 11.00, 12.00, 16.00, 18.00 and 20.00 h, and serum concentration of LH was measured by RIA. There was no significant fluctuation over time in the mean serum LH values for each sampling time in the group of animals implanted with empty cannulae in the MPO. In the group of animals implanted with CCK-8 into the MPO, the fluctuation of the mean LH values over time was significant, with a marked and significant elevation of LH values peaking at 16.00 h.

It was strongly suggested that CCK-8 was capable of stimulating LH-RH release, acting at the MPO.

Since the discovery of a gastrointestinal hormone, cholecystokinin (CCK) in the brain, many studies have been directed at finding the functional significance of this peptide in the brain (Dockray, 1976, Muller *et al.*, 1977). Concerning the influence on the secretion of anterior pituitary hormones, it has been reported that an intraventricular injection of CCK-8 in the ovariectomized rat produced a decline of plasma luteinizing hormone (LH) but elevated prolactin levels (Vijayan *et al.*, 1979). In the same study, plasma growth hormone levels rose but TSH levels fell. It was further reported that an intraventricular injection of CCK-8 induced an elevation of plasma

corticosterone levels (Itoh *et al.*, 1979, Fekete *et al.*, 1981).

On the basis of these findings, we have attempted to obtain more strict evidence supporting the role of CCK in the control of LH secretion by observing the effect of CCK implants into a localized area of the brain.

Materials and Methods

Female Wistar rats weighing 250-300 g maintained in a light-controlled (lights on from 05.00 to 19.00 h) room had been ovariectomized and implanted with guide cannulae in the medial preoptic area (MPO) 3 weeks later. The cannulae were made of 0.75 mm outer diameter stainless steel tube and were stereotactically implanted unilaterally according to the atlas by Albe-Fessard *et al.* (1966) under anesthesia with pentobarbital sodium. They were fixed to the skull using dental cement. Two weeks after the implantation of the guide cannula, the animal was primed

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with 20 μ g of estradiol benzoate in sesame oil at 12.00 h. At 12.00 h on the third day after the estrogen priming, powder CCK-8 (sulfated form, Squibb Co.), which was kindly donated by Dr. F. Kobayashi (Shionogi Research Laboratories, Shionogi Co.), was implanted through the inner cannula which was made of 0.30 mm outer diameter stainless steel tube. The inner cannulae containing CCK-8 at their tips were prepared by gently tamping the tubes into a watch glass containing the CCK-8. Material on the outer surface of the tubing was carefully removed, and then cannula was inserted through the guide cannula into the MPO without anesthesia. The control animals were implanted with empty cannulae.

0.5 ml blood samples were taken through an intraatrial cannula of silastic tube placed under anesthesia with ether on the day before the experiment, and an equal volume of heparinized (10 U/ml) 0.9% saline was replaced. Samplings were done at 11.00, 12.00 just before the CCK-8 implantation, 16.00, 18.00 and 20.00 h. After the final bleeding at 20.00 h, the rat was anesthetized with pentobarbital sodium and perfused with 10% buffered formalin. The sites of implantation were microscopically identified in frontal sections of the brains.

Serum LH concentrations were measured with a radioimmunoassay kit supplied by the NIAMDD. The method was principally based on the double antibody procedure described in the instructions with the kit. The referential standard was NIAMDD rat LH RP-1, and the LH values are expressed in terms of NIH-LH-S1. The mean minimally detectable amount of LH (95% confidence limits of buffer controls) on 3 assays was 0.23 ± 0.12 (SD) ng/ml. The within- and between-assay variance for the 3 assays, which were calculated from the triplicate determinations in each assay for the pool of rat serum containing 2.62 ± 0.12 (SE) ng/ml ($n=9$), were 2.1% and 12.4%, respectively.

Two way analysis of variance and the multiple range test of Duncan were used to test the statistical significance of fluctuations over time in the mean LH values for each sampling time. Student's *t*-test was used to test the significance of difference between groups of animals implanted with inner cannulae with and without CCK-8 at a particular time.

Fig. 1. Diagram summarizing the sites of CCK-8 implantations. The areas of cannula tips with CCK-8, as well as those of empty cannula tips are shown on frontal sections modified from the atlas of Albe-Fessard *et al.* (1966). Abbreviations; apom: area preoptica medialis, Ca: Commissura anterior, CC: Corpus callosum, Cd: nucleus Caudatus, Ch: Chiasma opticum, Fx: Fornix, Gp: Globus pallidus, Put: Putamen, Sch: nucleus suprachiasmaticus, and so: nucleus supraopticus.

Results

The sites of the implantation of CCK-8, as well as of the implantation of empty cannula in the MPO are shown in Fig. 1. The mean ($\pm$ SE) values of serum concentration of LH for each sampling time are shown diagrammatically in Fig. 2.

In the group of animals in which empty cannulae were implanted in the MPO, the fluctuation of mean LH values over time was shown to be not significant by analysis of variance. In contrast, in the animals which were implanted with CCK-8 in the MPO, there was observed a marked eleva-

tion of serum LH levels 4–6 h afterwards. Analysis of variance revealed that the fluctuation of mean LH values over time was significant ($p < 0.01$), and multiple range test revealed that the levels at 16.00 and 18.00 h were significantly higher than those pre-implanted ($p < 0.01$). Since the level at 18.00 h was quite low compared with the level at 16.00 h ($p < 0.01$), it seemed that the elevation of LH had attained its maximum by 16.00 h. Further, as apparent in Fig. 2, differences in LH levels between the groups of empty cannulae and CCK-8 were significant at 16.00 h ($p < 0.001$) and 18.00 h ($p < 0.05$).

Fig. 2. Effect of CCK-8 implantation in the MPO on serum LH levels of ovariectomized estradiol-primed rat. Each point represents the mean and the vertical lines indicate the SE. Numbers in parentheses show number of animals in each group.

* $p < 0.01$ vs levels at 11.00 and 12.00 h. ** $p < 0.01$ vs levels at 16.00 h.

Discussion

The results of the present experiment show clearly that CCK-8 implanted in the MPO is capable of stimulating the release of LH-RH, and subsequently the release of LH. Certainly, this was unexpected and surprising, since Vijayan *et al.* (1979) have shown that either the third ventricular or the intravenous administration of CCK-8 produced a rapid and significant suppression of plasma LH levels which continued for at least 1 h.

However, this discrepancy in the CCK effects between the ventricular administration and the MPO implantation reminded us of a paradoxical effect of dopamine (DA) we had found previously. That is, DA implants in the MPO in the ovariectomized estradiol-primed rat did induce a significant release of LH (Kawakami *et al.*, 1975, 1979), despite controversy over the role of DA in the stimulation of LH release. It had been reported that DA was effective in stimulating LH release (Schneider and McCann, 1970), whereas an inhibitory effect was indicated by other studies (Fuxe *et al.*, 1975, Gnodde and Schuiling, 1976). We had also found that an intravenous injection of DA lowered serum LH levels in the proestrous rat (Kimura *et al.*, 1975). However, the finding that DA was effective in stimulating LH release when implanted in a localized area, the MPO, seemed to account for the discrepancy between the results of many studies concerning the role of DA; we assumed that DA would act at the dual levels, at the MPO and the median eminence, inducing opposite effects on LH release (Kawakami *et al.*, 1975, 1979.)

The present finding that implantation of CCK-8 in the MPO stimulated LH release and the finding by Vijayan *et al.* (1979) that intraventricular administration suppressed it appeared to be in the same relationship as the findings for DA. Namely, it

is very probable that CCK-8 administered intravenously may act at the median eminence and inhibit LH-RH release, whereas CCK-8 implanted in the MPO may act at that level and stimulate LH-RH release. It has been reported that all nuclei of the rat hypothalamus including anterior preoptic nuclei contained moderate concentrations of CCK-8, although there was a considerable gradient of concentration in the hypothalamus with the median eminence, ventromedial and arcuate nuclei being the highest (Beinfeld *et al.*, 1981, Vanderhaeghen *et al.*, 1980, Anhut *et al.*, 1983). In the guinea pig, CCK neurons were particularly numerous in the MPO (Larsson and Rehfeld, 1979). In addition, it has been shown that CCK receptors were present in the brain areas where CCK distributed (Saito *et al.*, 1980). Those chemical findings would support our concept of the dual regulation of CCK-8 concerning LH release; i.e., at the MPO and the median eminence.

In relation with the very similar effects on LH release exerted by the CCK-8 and DA, recent studies have produced the interesting finding that some mesencephalic DA neurons projecting mainly to the limbic areas contained a CCK-8-like peptide (Hökfelt *et al.*, 1980). Although no evidence has been obtained that other DA neuron systems, such as the incerto-hypothalamic DA system which has been shown to send an abundance of fibers to the preoptic anterior hypothalamus (Björklund *et al.*, 1975), had CCK, it is possible that CCK and DA, which are contained in the same neuron, act synergistically, or at least, interact with each other, in carrying out their physiological functions. In support of this theory, Hökfelt *et al.* (1980) also demonstrated that CCK administered intraventricularly reduced DA turnover in some of the mesolimbic DA neurons that have been shown to contain CCK-8-like peptide. This speculation appears very attractive and thus our next subject is to obtain the evidence.

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