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Effects of Intracranially Implanted Cholecystokinin and Substance P on Serum Concentrations of Gonadotropins, Prolactin and Thyroid Stimulating Hormone in the Rat

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ABSTRACT—A cannula containing a mixture of cholecystokinin-4 (CCK-4) and cholesterol (1:1 or 5:3 by weight) and one containing a mixture of substance P (SP) and cholesterol (1:1) were implanted into the median eminence of male rats. CCK-4 reduced serum LH but not FSH, PRL and TSH concentrations; SP reduced serum TSH but not LH, FSH and PRL concentrations, according to measurements made 4–5 days following the implantations. CCK-4 implanted into the ventromedial or arcuate nucleus failed to have any effect on serum LH concentration. It is thus concluded that CCK-4 inhibits the release of LH, and SP inhibits the release of TSH, probably by inhibiting LHRH and TRH secretion from their respective axon terminals in the median eminence.

INTRODUCTION

The presence of the gastrin-cholecystokinin family of peptides (G-CCK) and substance P (SP) has been demonstrated by radioimmunoassay (RIA) in the hypothalamus of mammals (G-CCK: [1, 2]; SP: [3]) and pigeons (SP: [4]). Furthermore, the axon terminals of these substances have been shown immunohistochemically in the median eminence (ME) of mammals (G-CCK: [5]; SP: [6, 7, 10]) and lower vertebrates (G-CCK: [8]; SP: [9–11]). Thus, G-CCK and SP may possibly be essential to the regulation of the secretion of hypothalamic releasing or release-inhibiting hormones from the ME. To gain confirmation of this, various studies have been conducted on the effects of G-CCK and SP on the release of adenohypophysial hormones. Intraventricular injections of COOH-terminal octapeptide of CCK (CCK-8) [12] and gastrin [13] inhibited release of luteinizing hormone (LH), but CCK-8 stimulated it when implanted in the medial preoptic area in rats [14]. Further, intraventricularly injected SP stimulated LH and prolactin (PRL) secretion in rats [15] but had no influence on the release of LH or follicle-stimulating hormone (FSH) in the rhesus monkey [16]. It is thus apparent that these findings are at variance with each other.

Intraventricularly administered substances diffuse into nervous tissue and affect neurons near the ventricle, thus making it difficult to identify the site of action of the substances. In the present study, in order to find a particular site that is affected, a cannula containing the COOH-terminal tetrapeptide of CCK (CCK-4) or SP was chronically implanted into the ME. In addition, a cannula containing CCK-4 was implanted into the arcuate (AR) or hypothalamic ventromedial (HVM) nucleus, since G-CCK is found in these nuclei [2, 5, 21]. Then the serum concentrations of LH, FSH, PRL and thyroid-stimulating hormone (TSH) were estimated by RIA.

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MATERIALS AND METHODS

Male rats of the Wistar-Imamichi strain, 8 to 10 weeks of age (220–290 g), were used. The animals

were housed in an air-conditioned room at about 24°C under a 12 hr photoperiod (07:00–19:00) and had free access to water and food obtained from a commercial source. CCK-4 and SP (both from Peptide Institute, Inc.) were used for the implantation. CCK-4 was chosen among G-CCK for implantation, since it is known that CCK-4 interacts with CCK receptors in the rat brain [17]. For their implantation into the brain, CCK-4 and cholesterol were mixed well in a small glass-mortar with a pestle at a ratio of 1:1 or 5:3 by weight. SP and cholesterol were mixed in the same way at a ratio of 1:1. About 7–10 mg of each mixture or cholesterol alone was packed into a steel cannula of 0.35 mm in inner diameter. Each rat was anesthetized with Nembutal and fixed to a stereotaxic instrument. A cannula was then implanted stereotaxically into the brain, guiding its tip so that it would contact the desired hypothalamic sites, with the aid of X-rays. In the ME, the tip of a cannula containing CCK-4, SP or cholesterol was placed to contact with or just beneath the ependymal layer (Fig. 1). Furthermore, a cannula containing CCK-4 was implanted so that its tip would be in the center of the right AR or HVM (Fig. 1). It has already been demonstrated that substances mixed with cholesterol packed into a cannula, which is then implanted into the brain, diffuse out from the tip of the cannula for a certain period of time [18–20]. Rats implanted with a cannula containing either cholesterol or a piece of nylon thread of 0.3 mm in diameter served as cholesterol or blank controls. They were implanted into the same sites as those of the experimental rats. Animals without any implantation were also killed to serve as intact controls. The details of the implantation technique have been described in an earlier paper [19]. Four or 5 days after the operation, the rats were decapitated and blood samples were collected from the trunk between 10:00 and 11:00. Serum was separated by centrifugation at 2,000 rpm for 20 min and stored at −80°C until hormone assay. At the time of decapitation, a piece of hypothalamic tissue hit by the tip of the cannula was dissected out and fixed in Bouin's fluid. Paraffin sections were cut at 14 μm in thickness and stained with hematoxylin and eosin. The loci of the cannula tips in the hypothalamus were examined microscopically. Data were collected only from rats in which the cannula tip was situated at the desired position. Serum concentrations of LH, FSH, PRL and TSH were determined in triplicate with RIA kits supplied by the National Hormone and Pituitary Program. Reference standards for the assays were NIADDK rat LH-RP-1, rat FSH-RP-2, rat PRL-RP-1 and rat TSH-RP-1. Concentrations of these hormones were expressed in terms of LH-NIH-S1, FSH-RP-2, PRL-RP-1 and TSH-RP-1, respectively. The data were statistically analyzed by the Student's *t*-test and Cockran-Cox method.

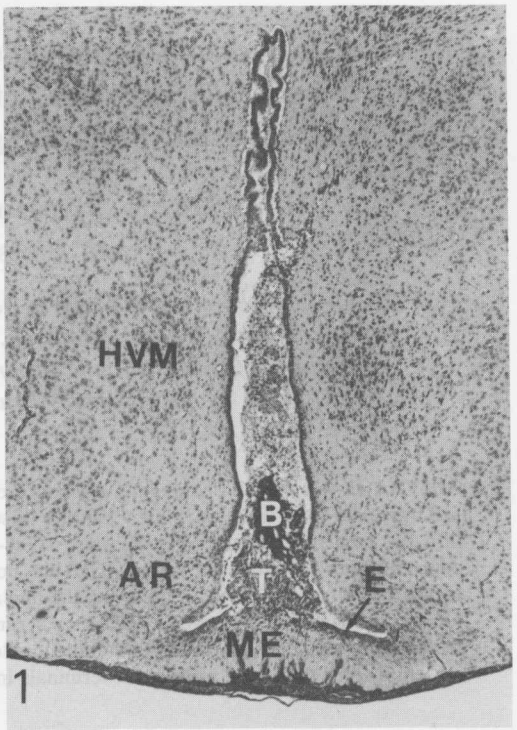

FIG. 1. Frontal section of basal hypothalamic region of a rat implanted with a cannula containing cholesterol into the median eminence (ME). The tissue was fixed in Bouin's fluid after the cannula was removed. The tip of the cannula was placed just beneath the ependymal layer. AR: arcuate nucleus, B: blood, E: ependymal layer, HVM: hypothalamic ventromedial nucleus, T: a tissue mass consisted mostly of ependymal, glial and some phagocytic cells. ×45.

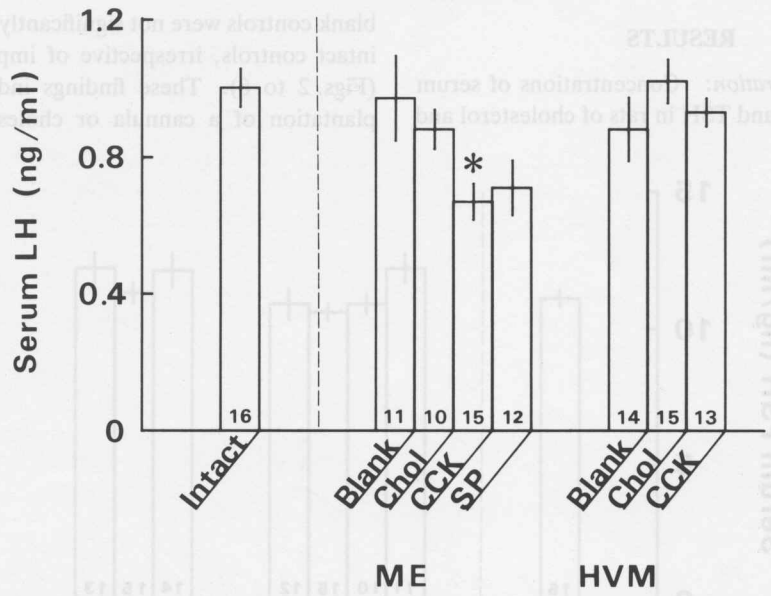

FIG. 2. Serum LH concentrations 4 or 5 days following the implantation of a cannula containing a piece of nylon thread (Blank), cholesterol (Chol), a mixture of CCK-4 and cholesterol at 5:3 by weight (CCK) or a mixture of SP and cholesterol at 1:1 (SP). Each column with vertical line shows mean and SE. Number of rats of each group is shown at the bottom of the column. ME: median eminence, HVM: hypothalamic ventromedial nucleus. * Significant ($p<0.05$) compared with the cholesterol control rats (Student's *t*-test).

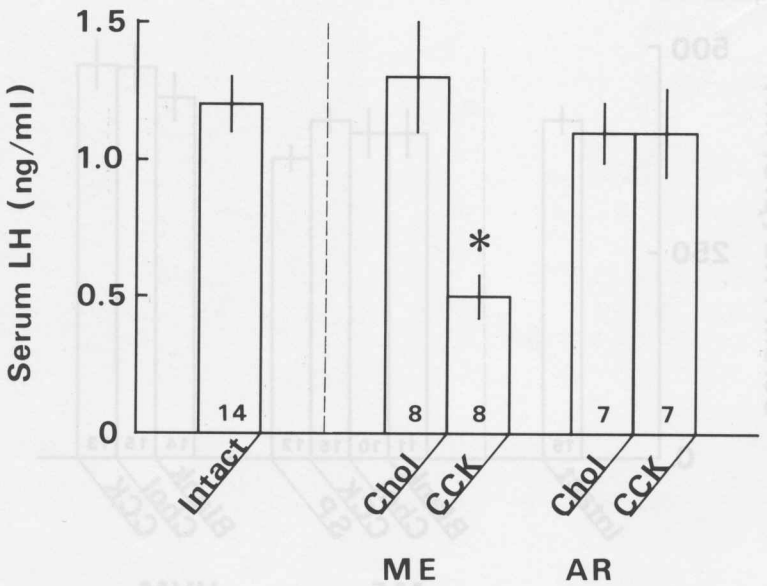

FIG. 3. Serum LH concentrations 4 or 5 days following the implantation. AR: arcuate nucleus. In this experiment, a mixture of CCK-4 and cholesterol at 1:1 (CCK) was used. Other abbreviations are the same as those in Fig. 2. * Significant ($P<0.05$) compared with the cholesterol control rats (Cockran-Cox method).

RESULTS

Effects of operation: Concentrations of serum LH, FSH, PRL and TSH in rats of cholesterol and blank controls were not significantly different from intact controls, irrespective of implantation sites (Figs. 2 to 6). These findings indicate that implantation of a cannula or cholesterol into the

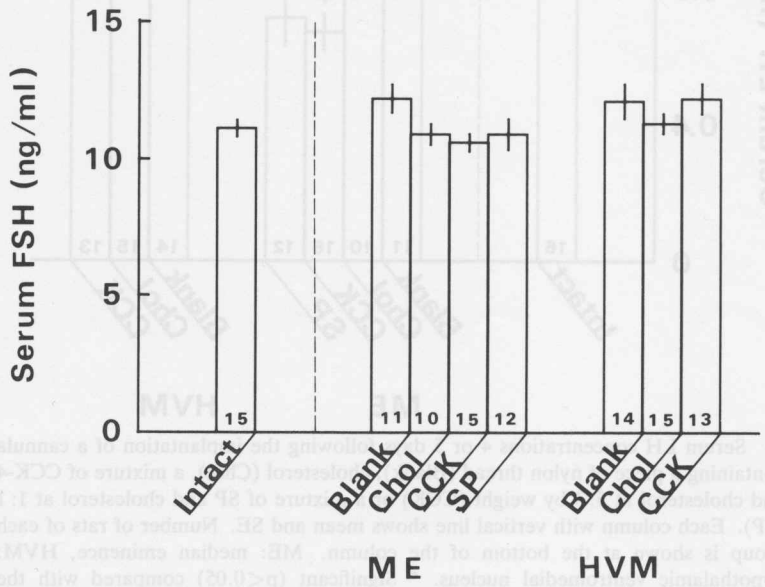

FIG. 4. Serum FSH concentrations 4 or 5 days following the implantation. Abbreviations are listed in Fig. 2. No effects were observed on FSH levels by any implantation.

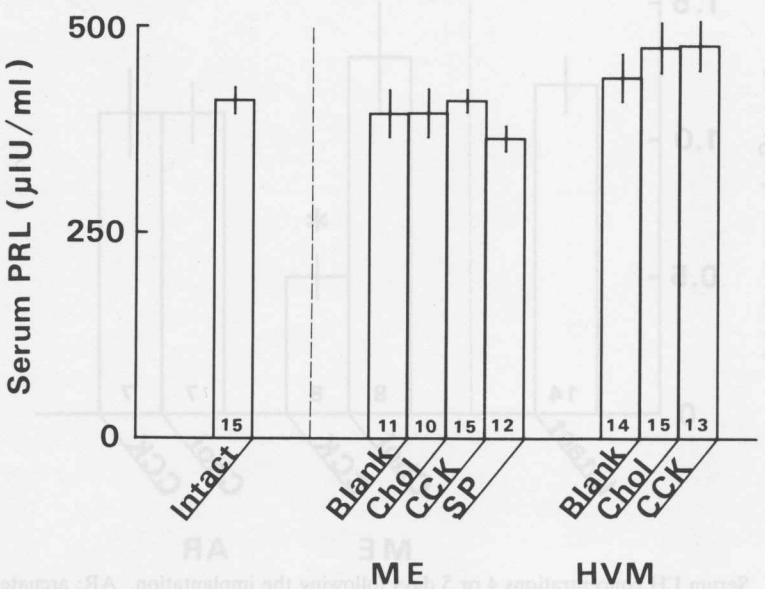

FIG. 5. Serum PRL concentrations 4 or 5 days following the implantation. Abbreviations are the same as those in Fig. 2. No effects were observed on PRL levels by any implantation.

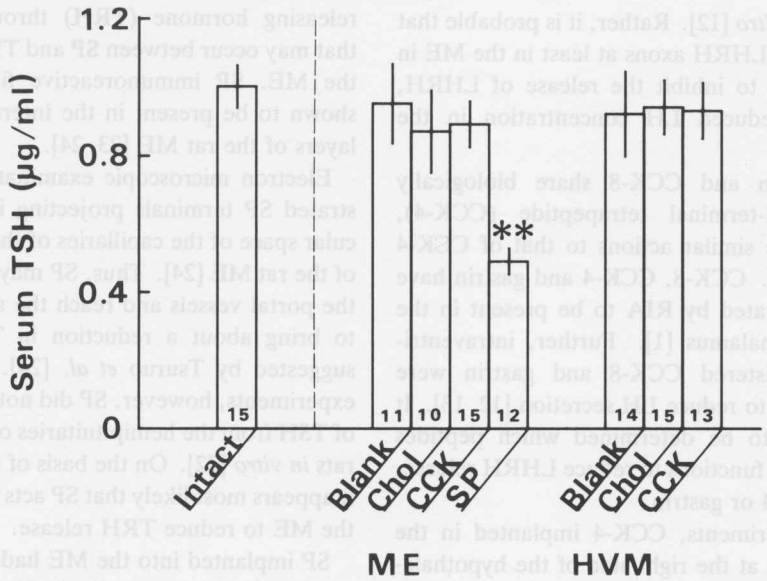

FIG. 6. Serum TSH concentrations 4 or 5 days following the implantation. Abbreviations are the same as those in Fig. 2. ** Significant ($P<0.01$) compared with the cholesterol control rats (Student's *t*-test).

brain has no effect on the release of LH, FSH, PRL and TSH. Therefore, data obtained from experimental rats were compared only with cholesterol controls.

Serum LH concentration: Two series of experiments were carried out: (1) implantation of CCK-4 plus cholesterol (5:3) in the ME and HVM (Fig. 2) and (2) implantation of CCK-4 plus cholesterol (1:1) in the ME and AR (Fig. 1). The serum LH concentration of rats implanted with CCK-4 into the ME was lower than that of cholesterol controls ($P<0.05$) (Figs. 2 and 3). Implantation of CCK-4 into the HVM (Fig. 2) or AR (Fig. 3) has no effect on serum LH concentration. In rats implanted with SP in the ME, the serum LH concentration was not significantly different from that of cholesterol controls (Fig. 2).

Serum FSH and PRL concentrations: Implantations of CCK-4 plus cholesterol (5:3) in both the ME and HVM and SP in the ME caused no change in the serum concentrations of FSH and PRL, compared with cholesterol controls (Figs. 4 and 5).

Serum TSH concentration: The implantation of a cannula containing CCK-4 plus cholesterol (5:3) had no effect on the concentration of serum TSH, whereas SP implantation into the ME reduced remarkably the serum TSH level ($P<0.01$), compared with cholesterol controls (Fig. 6).

DISCUSSION

In the present study, serum LH concentration decreased when CCK-4 was implanted into the ME. Vijayan *et al.* [12] reported that LH secretion was inhibited by CCK-8 injected intraventricularly in ovariectomized rats. This reduction of LH secretion may possibly be explained by the assumption that CCK-8 acted on the ME. This explanation is also supported by the observation that CCK-immunoreactive fibers are present in the ME [5].

Two possible mechanisms may be considered for the inhibitory effects of CCK-4 implanted in the ME on serum LH. (1) CCK-4 affects nerve terminals containing LH-releasing hormone (LHRH) in the ME causing reduced LHRH secretion, and (2) CCK-4 drains into the capillaries of the primary plexus, reaching the adenohypophysis to inhibit LH release. However, the second possibility seems unlikely, since CCK-8 had no effect on the release of LH from the hemipituita-

ries of rats *in vitro* [12]. Rather, it is probable that CCK acted on LHRH axons at least in the ME in such a way as to inhibit the release of LHRH, resulting in reduced LH concentration in the serum.

Since gastrin and CCK-8 share biologically active COOH-terminal tetrapeptide (CCK-4), they may have similar actions to that of CCK-4 within the ME. CCK-8, CCK-4 and gastrin have been demonstrated by RIA to be present in the porcine hypothalamus [1]. Further, intraventricularly administered CCK-8 and gastrin were actually found to reduce LH secretion [12, 13]. It thus remains to be determined which peptides physiologically functions to reduce LHRH release, CCK-8, CCK-4 or gastrin.

In our experiments, CCK-4 implanted in the HVM and AR at the right side of the hypothalamus had no effect on serum LH concentration, although the presence of many G-CCK immunoreactive fibers in the HVM [5] and relatively high concentration of G-CCK in the AR [2] have been reported. It is probable that the HVM and AR at the left side may have compensated for possible inhibition of LHRH release by CCK-4 or G-CCK in these regions may not be involved in the release of LHRH.

Serum concentrations of FSH, PRL and TSH were not affected by CCK-4 implantation in the HVM or ME of male rats. However, according to Vijayan *et al.* the intraventricular injection of gastrin reduced serum concentrations of PRL and TSH [13] and the intraventricular injection of CCK-8 reduced TSH secretion, but not FSH and PRL secretion [12] in ovariectomized rats. The differences between their results and ours may possibly be ascribed to differences in sites of action, duration of action, concentrations of substances administered and animal sex.

Implantation of SP into the ME caused a remarkable decrease in serum TSH in the present study. Vijayan and McCann [22], however, failed to observe such an inhibitory effect of intraventricular SP on TSH release in rats. This discrepancy may be explained by differences in experimental design, especially the mode of SP administration. The present data indicate SP implanted into the ME may possibly interfere with release of TSH-releasing hormone (TRH) through interactions that may occur between SP and TRH axons within the ME. SP immunoreactive fibers have been shown to be present in the internal and external layers of the rat ME [23, 24].

Electron microscopic examination has demonstrated SP terminals projecting into the perivascular space of the capillaries of the primary plexus of the rat ME [24]. Thus, SP may be released into the portal vessels and reach the adenohypophysis to bring about a reduction in TSH release, as suggested by Tsuruo *et al.* [24]. In the *in vitro* experiments, however, SP did not alter the release of TSH from the hemipituitaries of ovariectomized rats *in vitro* [22]. On the basis of the present data, it appears most likely that SP acts on TRH fibers in the ME to reduce TRH release.

SP implanted into the ME had no effect on the serum concentrations of LH, FSH and PRL in the present study. The absence of any effect of intraventricularly injected SP (100 μg) on the release of LH and FSH has been observed in normal female rhesus monkeys [16]. However, SP (2 μg) injected into the third ventricle stimulated LH and PRL secretion in ovariectomized rats [15]. Differences in reported data may be due to particular sites of action, dose amounts of SP and animal hormonal status. To achieve greater consistency in the results obtained, further experimentation on such aspects as implantations of mixtures of SP and cholesterol at different ratios should be carried out.

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