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Gastrin and cholecystokinin in pituitary neurons

(hormones/neuropeptides/radioimmunoassays/immunocytochemistry/coexistence)

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ABSTRACT **Gastrins occur in the hypothalamo-hypophyseal neurons of all mammalian species examined. In addition, human, bovine, and murine hypothalamo-hypophyseal neurons contain the homologous cholecystokinins (CCKs). CCK also occurs in neurons innervating bovine melanotrophs. Although the concentration of gastrin is of the same magnitude (15–30 pmol/g) in all neural lobes, the concentration of CCK varies from undetectable in pig and cat to 1 nmol/g in the cow. The constant occurrence of neurohypophyseal gastrin suggests a role different from that of the species-dependent CCK.**

The gut hormones gastrin and cholecystokinin (CCK) have a common origin (1) and a common COOH terminus (Trp-Met-Asp-Phe-NH_2), which constitutes their active site. Accordingly, they display the same spectrum of activities, although their potency towards the target organs depends on the different NH_2-terminal extensions. The gastrin–CCK family of peptides possesses the same general features and specificity problems as other families of homologous peptides, like the opioid peptides and oxytocin–vasopressin peptides.

Gastrin has now also been found in all lobes of the porcine pituitary (2), where its precursor, like pro-opiomelanocortin, is processed differently in corticotrophs and melanotrophs (3, 4). The porcine hypothalamo-hypophyseal system is the only known central gastrinergic neuronal system, whereas CCK is abundantly present in most remaining central nervous system regions (2–8).

We now report that though gastrin is constantly present in mammalian pituitary neurons, CCK also occurs in bovine, murine, and, to a lesser extent, human hypothalamo-hypophyseal neurons.

MATERIALS AND METHODS

Tissue Sampling and Preparation. Four pools each of 8–14 porcine pituitaries and of 8 bovine pituitaries were obtained at a local slaughterhouse 20–30 min postmortem. They were kept on ice until dissection. Three pituitaries were obtained from pentobarbital-anesthetized cats. Three pools of 16–44 rat pituitaries were dissected immediately postmortem. Three normal human pituitaries were obtained 6–16 hr postmortem. The porcine, feline, bovine, and murine glands were dissected into anterior, intermediate, and neural lobes under a microscope (×50, final magnification). Some bovine and the human anterior and neural lobes were dissected without magnification, but only the most anterior and posterior parts, respectively, were examined to ensure absence of contamination from other lobes. Further control was achieved by measuring the vasopressin and melanocyte-stimulating hormone (MSH) concentrations in the samples. Moreover, the secretory vesicles from two pools of rat neurointermediate lobes (n = 60 and 300) and one pool of bovine neural lobes (n = 6), isolated as described (9), were examined. All of the specimens were frozen in liquid nitrogen, minced, boiled for 20 min in redistilled water (pH 6.6, 5 ml/g of tissue), homogenized, and centrifuged and the supernatants were decanted. The pellets were reextracted in 0.5 M acetic acid, homogenized, and centrifuged. Both the neutral and acid supernatants were assayed in dilutions from 1:3 to 1:1000 for gastrin and CCK.

Chromatography. After radioimmunochemical measurement of the total gastrin and CCK concentrations, appropriate volumes (<1 ml) of both neutral and acid tissue or vesicle extracts were applied to calibrated Sephadex G-50 superfine columns (10 × 1000 mm). The columns were eluted at 4°C with 0.02 M barbital buffer (pH 8.4) at a flow rate of 4 ml/hr in fractions of 1.0 ml. The columns were calibrated with highly purified sulfated and nonsulfated porcine tetratriaconta- (gastrin-34), heptadeca- (gastrin-17), and tetradecapeptide (gastrin-14) gastrins (kind gifts from R. A. Gregory, Liverpool, U.K.) and, moreover, with highly purified sulfated porcine tritriacontapeptide CCK (CCK-33; a kind gift from V. Mutt, Stockholm, Sweden), highly purified synthetic sulfated octapeptide CCK (CCK-8; a kind gift from M. Ondetti, Princeton, NJ), and the COOH-terminal tetrapeptide amide common for gastrin and CCK (CCK-4; a kind gift from J. Morley, Cheshire, U.K.). The void and total volumes of the columns were determined with ^{125}I-labeled albumin and ^{22}NaCl, respectively. The pituitary gastrins from the gel chromatography were further purified by anion-exchange chromatography on aminoethyl (Whatman, AE-41) columns (10 × 150 mm). These columns were eluted at 20°C with a gradient of 0.05–0.2 M NH_4HCO_3 at a flow rate of 40 ml/hr in fractions of 1.5 ml. The pituitary CCK-8- and CCK-4-like material was also further purified on diethylaminoethyl (Whatman, DE-32) columns (10 × 150 mm) eluted as the AE-41 columns. To ensure identity with the gastrointestinal peptides, fractions from the ion-exchange chromatographies containing the bovine pituitary heptadecapeptide-like gastrins and octapeptide-like CCK were applied separately to reverse-phase HPLC for comparison with similar fractions from bovine antral and intestinal mucosa. The fractions were lyophilized and applied to a 8 × 250 mm column of C_{18} nucleosil silica eluted with a 10–60% CH_3CN gradient in 0.045% trifluoroacetic acid at 20°C and a flow rate of 1 ml/min. Fractions of 0.5 ml were collected, dried, reconstituted in 0.02 M barbital buffer (pH 8.4) containing 0.1% bovine serum albumin, and assayed.

Radioimmunoassays. Three sequence-specific assays were used in this study. The first assay using antiserum no. 2604 is specific for gastrin, with a negligible CCK reactivity (ID_{50} gastrin-17/ID_{50} CCK-8 < 0.001). It measures the precursor forms, component I and gastrin-34, with the same molar potency as gastrin-17. It also measures sulfated and nonsulfated forms with the same potency (10–12). The next assay using antiserum no. 2609 is directed against the common COOH terminus of gastrin and CCK. Hence, it measures all

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Abbreviations: CCK, cholecystokinin; Ab, antibody; MSH, melanocyte-stimulating hormone.

Table 1. Concentrations of gastrin and CCK in the pituitary lobes of various mammals

Mammal	*n*	Anterior lobe		Intermediate lobe		Neural lobe		Stalk	
		Gastrin	CCK	Gastrin	CCK	Gastrin	CCK	Gastrin	CCK
Pig	4*	1.9 (1.6–2.3)	0.1 (<0.1–0.2)	16.2 (4.3–29.3)	<0.1	22.4 (17.9–31.3)	<0.1	9.0 (6.4–13.7)	<0.1
Cow	8†	<0.1	0.2 (<0.1–0.8)	14.4 (3.1–60.2)	137 (40–211)	23.8 (16.8–34.0)	897 (522–1120)	13.3 (10.0–16.1)	288 (63–513)
Cat	3†	9.6 (1.5–19.0)	<0.1	40.9 (28.0–54.3)	<0.1	31.2 (23.1–42.7)	<0.1	14.8 (4.5–23.2)	<0.1
Rat	3‡	0.9 (0.7–1.0)	<0.1	3.6 (2.3–4.7)	0.8 (0.5–1.0)	15.3 (7.4–20.1)	264 (173–432)	—	—
Man	3†	1.1 (0.8–1.3)	0.9 (0.3–1.6)	—	—	13.1 (4.1–19.5)	39 (25–54)	5.7 (2.9–8.4)	12.8 (6.2–17.7)

Concentration of gastrin is given in pmol equivalents of porcine G-17 per g of tissue (wet weight) and concentration of CCK is given in pmol equivalents of porcine CCK-8 per g of tissue (wet weight). Values are expressed as the mean (range in parentheses).
*Each sample composed of tissue from 8 to 14 pituitary lobes.
†Each sample constituted by individual lobes.
‡Each sample composed of tissue from 16 to 44 pituitary lobes.

gastrins and CCKs irrespective of their degree of sulfation (11, 12). The third assay using antiserum no. 4698 is specific for CCK and does not measure any gastrins. It is directed against sequence 25–30 of CCK-33 and is entirely dependent on O-sulfation of tyrosyl in position 27. This assay measures sulfated CCK-33, CCK-12, and CCK-8 with equimolar potency (11). Tracers, standards, assay conditions, and reliability parameters have been described in detail (10–12).

Immunocytochemistry. For immunocytochemistry, either immersion-fixation (man, pig, cow) or perfusion-fixation (rat, cat) in 4% paraformaldehyde was used. Cryostat sections (3, 13, 14) were stained with antisera specific for the common COOH terminus of gastrin and CCK [antisera nos. 4562 and 2717 (10–12)] and a novel CCK-specific antiserum [antibody (Ab) 1561, recognizing a part of the 16–29 sequence of CCK-33] using peroxidase-antiperoxidase or indirect immunofluorescence techniques (13, 14). Antisera nos. 4562 and 2717 have the same specificity but a higher titer and consequently a lower background staining than antiserum no. 2609 (used in radioimmunoassay because of a higher avidity). The titer of antiserum no. 4698 (used in radioimmunoassay) has unfortunately proved too low to stain CCK neurons unambiguously. Controls included both conventional staining controls (13, 14) and absorption controls against (*i*) the synthetic common COOH-terminal tetrapeptide amide (CCK-4), (*ii*) synthetic human gastrin-17, and (*iii*) synthetic porcine CCK-(1–15) and highly purified porcine CCK-33.

RESULTS

As shown in Table 1, gastrin was present in the pituitary of all species examined. The concentrations in the anterior lobe, where gastrin previously has been shown to occur in

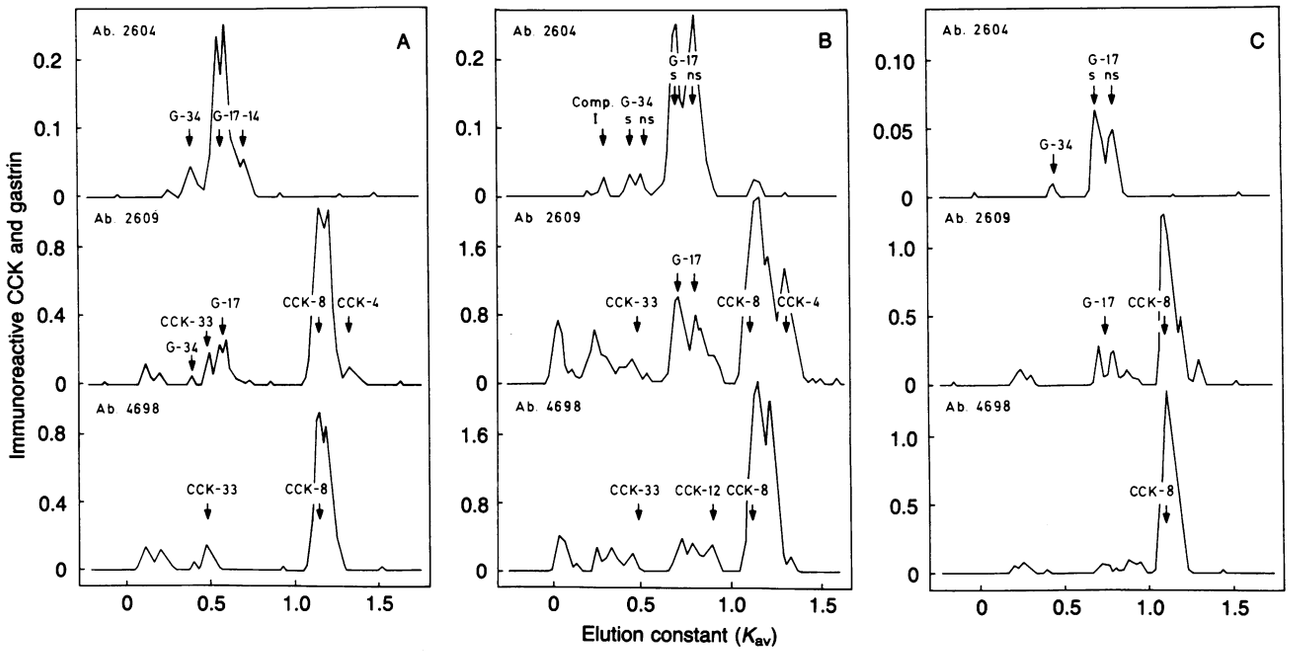

FIG. 1. Gel chromatography of extracts from murine neural lobe (*A*) and from bovine neural (*B*) and intermediate (*C*) lobes. After dissection the tissues were immersed in boiling water for 20 min (0.2 g/ml) and homogenized for 5 min. After centrifugation the pellet was reextracted with 0.5 M acetic acid and rehomogenized. The neutral and acid extracts were applied to calibrated Sephadex G-50 superfine columns (10 × 1000 mm) eluted at 4°C with 0.02 M barbital buffer (pH 8.4) at a flow rate of 4 ml/hr. The elutions were monitored by three different radioimmunoassays: Antiserum no. 2604 (upper panels) binds heptadecapeptide gastrin (G-17) and the larger molecular forms (component I and G-34), sulfated (s) as well as nonsulfated (ns), with equimolar potency. It crossreacts <0.1% with CCK-8. Antiserum no. 2609 (middle panels) is directed against the common COOH terminus and consequently binds both the gastrins and the CCKs. Antiserum no. 4698 (lower panels) is directed against the sulfated sequence 25–30 of porcine CCK-33. It binds CCK-8, CCK-12, and CCK-33 with similar potency. Immunoreactive CCK and gastrin are given on the ordinate as nmol equivalents of CCK-8 per liter and nmol equivalents of G-17 per liter, respectively.

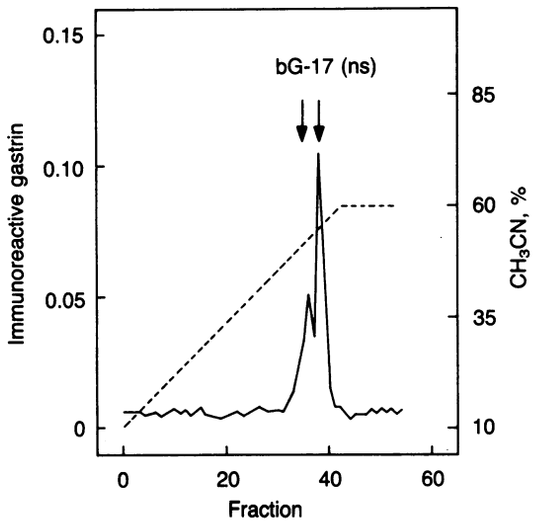

FIG. 2. Reverse-phase HPLC of nonsulfated heptadecapeptide gastrin from the neural lobe of the bovine pituitary. A boiling water extract (10 ml/g of tissue) was first subjected to Sephadex G-50 superfine chromatography (see Fig. 1). The fractions corresponding to heptadecapeptide gastrin were pooled, lyophilized, dissolved in 0.05 M NH_4HCO_3, and applied to an ion-exchange column (10 × 150 mm, AE-41 cellulose), gradient eluted with NH_4HCO_3 (0.05–0.4 M). The fractions corresponding to either sulfated or nonsulfated gastrin were pooled, lyophilized, and applied separately to a column (8 × 250 mm) of C_{18} nucleosil silica, which was eluted with the indicated CH_3CN gradient in 0.045% trifluoroacetic acid at room temperature and a flow rate of 1 ml/min. Fractions of 0.5 ml were collected and assayed with a gastrin-specific radioimmunoassay using antiserum no. 2604. The column was calibrated with nonsulfated heptadecapeptide gastrin isolated as described above from bovine antrum (arrows). As shown, the nonsulfated pituitary gastrin-17 eluted in the same biphasic positions as the antral nonsulfated gastrin-17. Immunoreactive gastrin is given on the ordinate as nmol equivalents of gastrin-17 per liter.

corticotrophs in pigs and cats (3), were generally one order of magnitude lower than in the intermediate and neural lobes. In the murine and bovine pituitaries, no immunoreactive cells were found in the anterior lobe. In the bovine anterior lobe the concentration of gastrin was below the detection limit of the assays. The gastrin concentrations in the intermediate and neural lobes varied little between the species.

Gel (Fig. 1) and ion-exchange (not shown) chromatography of neural lobe extracts showed that sulfated gastrin-17 and nonsulfated gastrin-17 were the dominant gastrin components in bovine, murine, and porcine (4) pituitaries. As illustrated for the bovine gastrin-17-like component, reverse-phase HPLC indicated that the gastrin/CCK peptides from the pituitary neural lobe were identical to the corresponding peptides extracted from the antral and intestinal mucosa (Fig. 2). The precursor forms, component I and gastrin-34, constituted <15% (Fig. 1). As previously shown (3), gastrin in the neural lobe is located in nerve terminals originating from the hypothalamus (Table 1).

Gel chromatography of extracts from carefully isolated bovine intermediate lobes shows that the predominating molecular forms are gastrin-17 and CCK-8, respectively (Fig. 1). Immunocytochemical studies (Fig. 3) have identified COOH-terminal gastrin/CCK immunoreactivity in endocrine α-MSH cells as well as in nerve endings terminating on these cells. The new sensitive CCK antiserum (no. 1561) stained CCK in nerve terminals of both the neural and intermediate lobes, whereas the endocrine intermediate lobe cells consistently failed to display CCK immunoreactivity. The staining encompassed most, but not all of the posterior pituitary axons. In no other species than ox have nerve endings been found in the intermediate lobe. In pigs, cats, dogs, and oxen the α-MSH cells all display strong, but cell-to-cell variable, COOH-terminal gastrin/CCK immunoreactivity. In the rat no such immunoreactivity was detected, presumably due to the lower gastrin concentration (Table 1). The immunocytochemical data combined with the measurements on extracts of the isolated intermediate and isolated neural lobes (Fig. 1) suggest that intermediate lobe α-MSH cells of the ox, as previously demonstrated in cats and pigs (3, 4), contain gastrin, whereas the neurons innervating the α-MSH cells contain CCK.

DISCUSSION

The present study shows that neurohypophyseal gastrin is a prominent feature of the mammalian pituitary, because it occurred in all species examined in concentrations of the same magnitude (Table 1). Although the pituitary gastrin concentrations are low in comparison with the concentrations in the antral mucosa, the consistent occurrence in constant amounts suggests that the hypothalamo-hypophyseal gastrin is of significance. The pituitary gastrins are not due to contamination from blood as they are 1000-fold above plasma concentrations (15). Besides, cat plasma contains no large molecular forms of gastrin [gastrin-34 and component I (16)], which, however, are present abundantly in cat pituitaries. Hence, gastrin is synthesized in hypothalamo-hypophyseal neurons, a conclusion further supported by the differential processing of the gastrin precursor in the different pituitary cell types (4).

In contrast to gastrin, the pituitary occurrence of CCK varied between species and pituitary lobes. Significant CCK immunoreactivity in anterior lobes was detectable only in humans (Table 1). Moreover, CCK was found in the neural lobe in cow and rat (Table 1). Here, the concentrations were higher than in other regions of the central nervous system (17–19). In contrast, CCK was undetectable in porcine and feline neural lobes (Table 1). CCK from the murine and bovine neural lobes was mainly the sulfated octapeptide, but substantial amounts of larger precursor forms also occurred—especially in the bovine neural lobe (Fig. 1). The high concentration of CCK probably explains the previously reported failure to detect gastrin in the rat pituitary (20). When only one antiserum directed against the common COOH terminus is used, the relatively small gastrin fraction (<10%, Table 1) is likely to drown in the CCK immunoreactivity.

Although gastrin and CCK occur together in the neural lobe of man, rat, and ox, it is not known whether the two peptides are synthesized in the same neurons. Using a COOH-terminal directed and hence crossreactive antiserum, Vanderhaeghen *et al.* have demonstrated that gastrin/CCK immunoreactivity in rat and cow is localized together with oxytocin–neurophysin in magnocellular hypothalamo-hypophyseal neurons (21). However, they could not distinguish gastrin from CCK. Such distinction requires antisera specific for unique sequences of CCK and gastrin. Because bovine and murine gastrin in these sequences differ from porcine and human gastrin, species-specific antisera are necessary, but not yet available. Notably, the present localization with specific CCK antibodies (antiserum no. 1561, which does not react with gastrin) to hypothalamo-hypophyseal neurons represents, to the best of our knowledge, the first specific demonstration of CCK neurons.

Because gastrin and CCK peptides seem to occur together with oxytocin and perhaps vasopressin, it has been speculated that gastrin or CCK (or both) in rat and cow might be part of a large multihormonal precursor (22). However, by recombinant DNA techniques, separate small gastrin precursors have recently been identified in porcine antral and human gastrinoma cells (23, 24). It now remains to be shown whether the pituitary cells translate the same precursor.

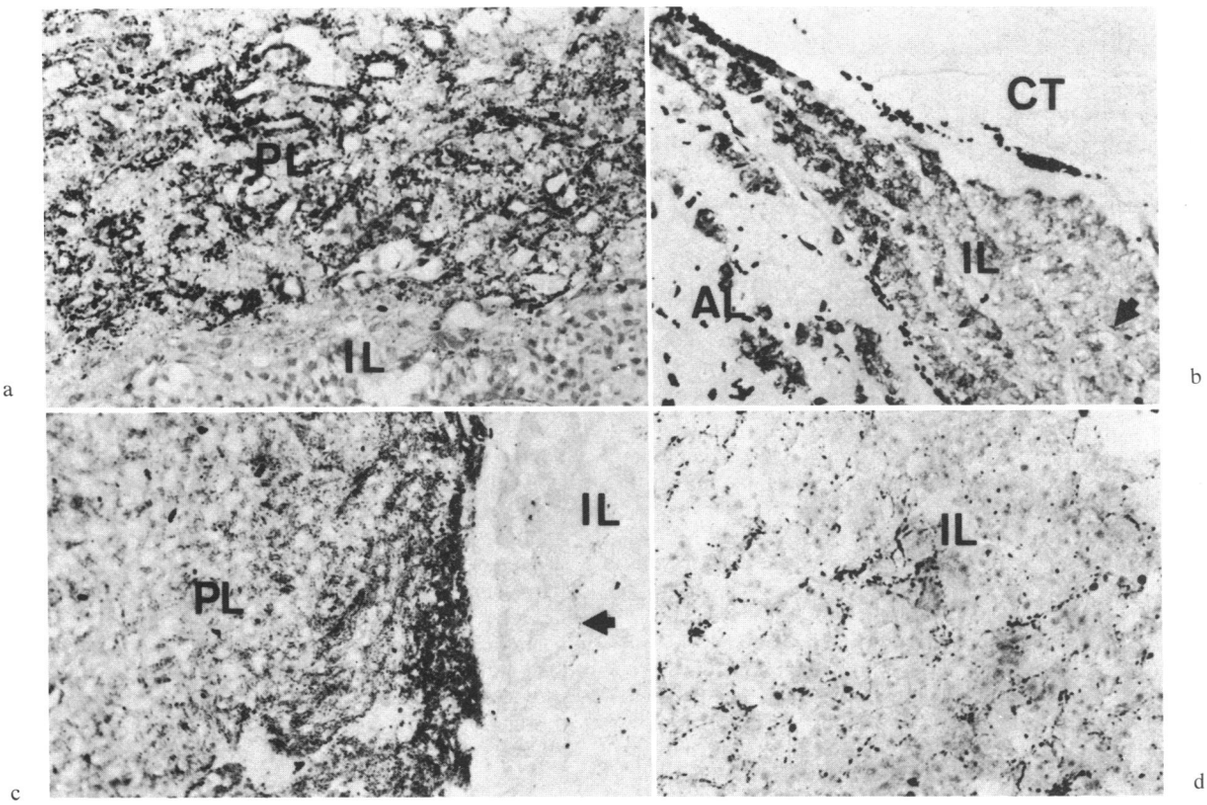

FIG. 3. Cryostat sections of paraformaldehyde-fixed pituitaries of rat (*a*) and cow (*b–d*) stained with the peroxidase-antiperoxidase technique using either Ab no. 4562, which recognizes the COOH-terminal tetrapeptide portion common to both gastrin and CCK (*a* and *b*), or the CCK-(15–29)-specific Ab no. 1561 (*c* and *d*), which does not react with gastrin. Note that in the rat pituitary (*a*) nerve terminals of the posterior lobe (PL) are strongly stained by Ab no. 4562, whereas the intermediate lobe (IL) cells only show background stain. In contrast, in the cow pituitary (*b*), the IL cells show variable (moderate to strong) staining. (*b*) Part of the IL with a few cells invading the anterior lobe (AL). Part of the IL is more weakly stained and here COOH-terminal immunoreactive nerve terminals can be discerned (arrow). CT indicates connective tissue of the sella turcica. (*c*) Part of the low PL and IL are included. Note that the numerous nerve terminals of the PL and the scattered nerve terminals of the IL react with the CCK-specific antiserum no. 1561 (arrow), whereas the MSH cells fail to react for CCK. (*d*) Close-up view of the CCK-immunoreactive terminals contacting the gastrin-containing α-MSH cells of the IL. (*a*, ×150; *b*, ×90; *c*, ×90; *d*, ×180.)

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