Table 2. Light and temperature influence on the visual pigment composition and REH activity in the goldfish

Group	Light and temperature conditions	Porphyropsin %	Specific activity (pmol retinol/mg/h) Retina	RPE/choroid
1	Room light 20 °C	100	105.6 ± 7.2 (n = 6)	118.4 ± 15.5 (n = 8)
2	Room light 30 °C	90	143.3 ± 9.6 (n = 8)	115.4 ± 20.3 (n = 8)
3	Dim light 16L/8D, 30 °C	30	223.5 ± 11.5 (n = 18)	146.8 ± 33.2 (n = 18)

Upon arrival from the supplier, goldfish were acclimated for 30 days in the specified conditions (for details of these light and temperature conditions)[9] before visual pigment (percent porphyropsin: mean value from two fish) and REH (mean ± SD, n = number of samples) analyses.

light and at room temperature for 30 days) were comparable to those (table 1) obtained from fish upon arrival. This suggests that REH activity in these animals remained unchanged during the 30-day duration of the experiment. Likewise, the composition of photopigments in the retina remained high in porphyropsin proportions in fish held under room conditions.

Although light and temperature did not exert a significant influence on the REH activity in the RPE of the eye (table 2), these environmental factors affected both visual pigment composition as well as REH activity in the retina (table 2). An increase in temperature (compare results from groups 2 to 1) associated with a 40% increase in REH activity in the retina but little change in photopigment composition. A combined effect of light and temperature (compare results from groups 3 to 1) resulted in a large reduction of porphyropsin proportion (from 100% to 30%, which is in agreement with our earlier findings)[9] and a significant increase (Student's t-test, $p \geq 0.001$) in the REH activity.

The effect of temperature acclimation on the enzyme activity has been well established in the literature[16]. For example, the K_m values of acetylcholinesterase in rainbow trout acclimated to 2 °C and to 18 °C were completely different when it was assayed at 5, 10, 15 and 20 °C. Examples of these 'thermal modulations' are also shown in other enzymes such as phosphoenolpyruvate, lactate dehydrogenase (and others) and in other species such as rabbit, tuna (and others)[16]. Data from the present study show that light and temperature give rise to a significant change in the ocular REH activity in goldfish. Although the present study has not elucidated whether light/temperature had influenced either the K_m or the V_{max} of the REH, this light/temperature induced increase in the ocular REH activity will clearly increase the rate of release of retinol in the eye, leading to an increase in the availability of retinal chromophore for the formation of rhodopsin. Furthermore, it is also important to study whether this REH activity is specific for retinyl ester in comparison to 3,4-didehydroretinyl ester in order to establish the significance of this control mechanism.

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The influence of different nutrients on plasma cholecystokinin levels in the rat[1]

B. R. Douglas, R. A. Woutersen*, J. B. M. J. Jansen, A. J. L. de Jong and C. B. H. W. Lamers

*Department of Gastroenterology and Hepatology, University Hospital Leiden, Bldg. 1 C4-P, P.O. Box 9600, NL-2300 RC Leiden (The Netherlands), and *Department of Biological Toxicology, T.N.O.-CIVO Toxicology and Nutrition Institute, P.O. Box 360, NL-3700 AJ Zeist (The Netherlands), 8 May 1987*

Summary. Isocaloric and isovolemic amounts of protein (casein), fat (intralipid) and carbohydrate (saccharose) and an isovolemic control solution of water were administered intragastrically to conscious rats. The plasma CCK levels, determined by a sensitive and specific radioimmunoassay, showed an increment of 6.3 ± 0.6, 2.7 ± 0.5, 1.7 ± 0.4 and − 0.9 ± 0.4 pM, respectively (basal value 2.5 ± 0.3 pM). The threshold increment of plasma CCK to stimulate pancreatic enzyme secretion by exogenous CCK was found to be 1.5 pM. It is therefore concluded that casein is a potent stimulus for CCK secretion and pancreatic secretion, but that fat and even carbohydrate, although less potent, also produce a CCK increment above the threshold for pancreatic secretion.

Key words. Rats; nutrients; cholecystokinin; pancreatic secretion.

The cells producing the gut hormone cholecystokinin (CCK) are localized in the proximal small intestine and release CCK into the circulation following ingestion of food. CCK is believed to be the most important regulator of pancreatic secretion, and it is assumed that those nutrients that stimulate pancreatic enzyme secretion do so by stimulating CCK release.

It has previously been demonstrated that ingestion of peptides or long chain triglycerides stimulates CCK release in man[2], while other studies have shown that peptides, amino acids and monoglycerides stimulate pancreatic enzyme secretion in human subjects[3,4]. In the rat, protein in the form of casein has been shown to stimulate pancreatic secretion[5,6], whereas hydrolyzed protein and amino acids do not[7].

Relatively few studies have been published concerning the effects of fat or carbohydrate on the rat pancreas[8-10]. In rats the role of CCK in pancreatic secretory response has only been indirectly demonstrated, using a bioassay[11]. From these studies it appeared that casein and trypsin inhibitors have stimulatory effects on CCK release whereas fats, carbohydrates and amino acids do not[10,12]. Because the rat is frequently used as an animal model to study pancreas physiology and pathology it is of the utmost importance to detect possible differences between this species and man in response to nutrients.

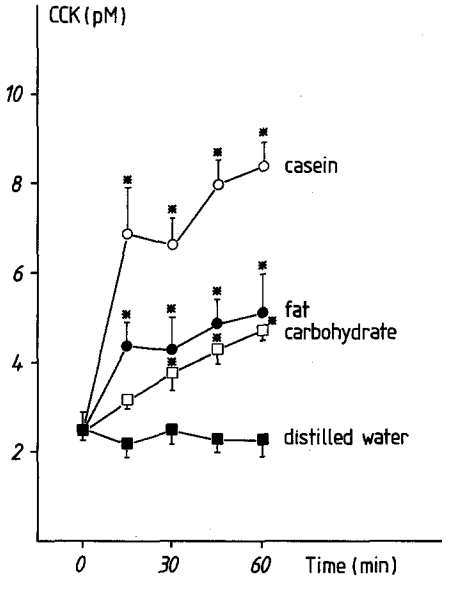
For this reason we studied the stimulatory effects of different nutrients on CCK release in the rat using a highly specific and sensitive radioimmunoassay.

Materials and methods. Isocaloric amounts of the following nutrients were studied: casein (CHV, Veghel, Netherlands), saccharose (Sigma Chemical St Louis, MO, USA), Intralipid (an emulsion containing 20% highly purified soybean oil from Kabi Vitrum, Stockholm, Sweden). Distilled water was added to the different nutrients to achieve an equal volume of 5 ml. Adult male Wistar rats weighing approximately 300 g (from Winkelmann, West Germany) were maintained on a 12 h light/12 h dark cycle and fed standard rat chow. They were kept in separate cages and in a fasting state overnight before the study. Four groups of 10 rats were fed either 5 ml of isocaloric amounts of casein, carbohydrates, fat or 5 ml distilled water via an oro-gastric tube inserted into the stomach. Blood was obtained before, and 15, 30, 45 and 60 min after administration of the test meal, and collected in iced heparin-treated tubes. Thereafter the blood specimens were centrifuged at 4 °C for 10 min to obtain the plasma. The threshold plasma CCK increase necessary for stimulation of pancreatic protein secretion was determined in 6 rats by infusion of doubling doses of CCK-33, from 1 to 16 IDU/kg, in conscious animals with a cannulated pancreatic duct.

Plasma CCK levels were measured by a highly sensitive and specific radioimmunoassay[2] known to bind to rat CCK[13], and pancreatic protein was assayed by the Lowry method[14]. Antibody T 204, raised in a rabbit against CCK-33 coupled to bovine serum albumin, was used in the CCK-assay. The antibody reacts to sulphated carboxy-terminal CCK-peptides and, therefore, quantitates all biologically active forms of CCK[2]. CCK-33 coupled to ^{125}I-hydroxyphenylpropionic acid succinimide ester (Bolton-Hunter reagent) was used as label and separation between bound and free peptide was achieved by adsorption of the unbound peptide to a charcoal suspension. The intra-assay coefficient of variation ranged from 4.6–11.6% and the interassay coefficient of variation was between 11.3 and 26.1%. The detection limit of the assay was 0.5 pM. The increment was calculated by subtraction of basal value from the peak postprandial level. Integrated plasma CCK secretion was determined by calculation of the area under the plasma concentration–time curve after subtraction of the basal value. Results were expressed as the mean ± SEM. Statistical analysis of plasma CCK responses between test meals was performed using a two-way analysis of variance. When the analysis indicated that the various diets elicited different responses, paired Student's t-tests were used to compare responses between two test meals.

Results and discussion. Casein, fat and carbohydrate all produced plasma CCK levels that were significantly higher than those found after 5 ml of distilled water, which showed no increase whatsoever above basal value (2.5 ± 0.3 pM) (fig. 1). Casein was by far the most potent stimulus for CCK secretion, giving rise to an increment of 6.3 ± 0.6 ($p < 0.001$) (fig. 1) and an integrated response of 245 ± 30 pM.60 min ($p < 0.001$). The fat emulsion caused an increment of 2.7 ± 0.5 pM ($p < 0.001$) (fig. 1) and an integrated response of 99 ± 4.0 pM.60 min. The increase after saccharose administration did not become significant until 30 min but showed a final increment of 1.7 ± 0.4 pM ($p < 0.01$) (fig. 1), whereas the integrated response in this case was still 72 ± 11 pM.60 min ($p < 0.01$); the control solution did not change plasma CCK (increment − 0.9 ± 0.4 pM, integrated secretion − 44 ± 9 pM.60 min). The threshold for pancreatic secretion during infusion of exogenous CCK was found to be 1.5 pM.

This is the first time the effect of isocaloric amounts of different nutrients on CCK release in the rat has been compared by means of CCK determination with a highly specific and sensitive radioimmunoassay. This study confirms the results of previous studies using bioassays, that unhydrolyzed protein is the most potent food stimulant of CCK release and thus pancreatic enzyme secretion in the rat[5-7,10,12]. Plasma CCK concentrations rapidly and steadily increased until 60 min after administration of the stimulus. In contrast to other studies[10], tendency to decrease was not observed. The present study also showed quite a rapid and statistically significant increase of CCK after administration of a triglyceride emulsion consisting of mainly long chain unsaturated triglycerides (soybean oil). This is especially interesting since Laugier and Sarles[8] and Demol and Sarles[9] found an initial increase in pancreatic protein output after intraduodenal injection of oleate and olive oil. The present study suggests that this increase could very well have been mediated by CCK release, since the rise in plasma CCK level in response to fat exceeded the threshold for pancreatic stimulation.



Plasma CCK responses to intragastric administration of different nutrients (n = 10). Asterisks denote significant increases above basal values ($p < 0.05$).

Also of interest is the slight, but statistically significant increase in CCK plasma levels after intragastric administration of a 10% saccharose solution. The increases in plasma CCK after the 3 nutrients cannot be explained by distention of the proximal small intestine as the control group with distilled water showed no effect on CCK levels.

It has been proposed that a negative feedback mechanism regulates pancreatic secretion in rats. Intraluminal trypsin in the proximal small intestine is assumed to play a central role in this mechanism, presumably by inhibiting CCK release, resulting in a decrease in pancreatic enzyme secretion [14]. The finding that feeding of trypsin inhibitors causes a significant and sustained increase in plasma CCK levels and thereby an increase in pancreatic secretion supports this hypothesis [12, 14, 16].

Our study demonstrates that, although intraluminal trypsin activity may play an important role in the regulation of pancreatic enzyme secretion, other mechanisms of CCK release do play a role in the postprandial stimulation of the pancreas in rats. Triglycerides and oligosaccharides give rise to a plasma CCK increment above the threshold for stimulation of pancreatic secretion, even though they do not possess any trypsin inhibiting activity as casein does [15].

The results of the present study lead to the following conclusions: 1) Casein, an unhydrolyzed protein, is a potent stimulus for CCK release in the rat. 2) Triglycerides, although less potent, still produce a CCK increment above the threshold for stimulation of pancreatic secretion. 3) Oligosaccharides also produce a CCK increase above this threshold. 4) These effects are not due to distention of the stomach or proximal small intestine.

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Influence of age on epidermal growth factor receptor level in the rat brain

M. Hiramatsu, M. Kashimata, A. Sato, M. Murayama and N. Minami

Department of Dental Pharmacology, Josai Dental University, Sakado, Saitama 350-02 (Japan), 24 August 1987

Summary. The influence of age on ^{125}I-epidermal growth factor (EGF) binding to rat brain plasma membranes was investigated. The specific binding of EGF to membranes decreased gradually with age in both male and female rats. There was no significant difference in the specific binding between males and females. Scatchard analysis of the binding data showed that the decrease in EGF binding with age was due to a decrease in the number of EGF receptors.
Key words. Age; epidermal growth factor receptor; brain; rat.

Epidermal growth factor (EGF) is a single chain polypeptide of 53 amino acids, first isolated from male mouse submandibular gland [1] and subsequently from human urine [2]. This peptide is a potent mitogen and differentiation factor for a variety of cells both in vivo and in vitro . Like other polypeptide hormones, EGF binds to a specific plasma membrane receptor in target cells [4]. It has been generally accepted that one of the earliest signals of EGF action is the autophosphorylation of EGF receptor following the activation of protein kinase integrated in the receptor molecule [5]. The EGF receptor is known to be distributed in various organs including the liver, skin, and gastrointestinal tract [4,6]. Moreover, a recent report has demonstrated the presence of EGF receptor in the brain of rabbits [7]. In this study, we investigated the influence of age on EGF receptor level in the rat brain.

Materials and methods. EGF was isolated from male mouse submandibular glands by the method of Savage and Cohen [8]. ^{125}I-EGF was purchased from New England Nuclear, Boston, MA. Phenylmethylsulfonyl fluoride (PMSF) and leupeptin were purchased from Sigma Chemical Co., St. Louis, MO.

Male and female Wistar rats of different ages were killed by ether anesthesia, and their whole brains were removed. The brain from each animal was homogenized in an all-glass homogenizer with 30 vol of 1 mM $NaHCO_3$ containing 2 mM PMSF and 10 μg/ml leupeptin. The homogenate was centrifuged for 10 min at 600 × g, and the supernatant obtained was then centrifuged for 30 min at 20,000 × g. The resulting pellet was washed once with the 1 mM $NaHCO_3$ solution described above, suspended in the incubation buffer (100 mM HEPES, pH 7.4, containing 120 mM NaCl, 1.2 mM $MgSO_4$, 2.5 mM KCl, 15 mM $NaC_2H_3O_2$, 10 mM glucose, and 1 mM EDTA), and used as the membrane preparation. Protein content in the sample was measured by the method of Lowry et al. [9].

The components of the binding assay consisted of ^{125}I-EGF (1 or 4 nM) and membrane preparation (500 μg protein) in 0.15 ml of the incubation buffer containing 1% bovine serum albumin (BSA). The medium was incubated at 24 °C for 90 min with or without an excess of unlabeled EGF (2000 nM), and then centrifuged for 5 min at 20,000 × g. The resulting pellet was washed once with 0.5 ml of the incubation buffer containing 1% BSA, and radioactivity in the