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HYPOTHALAMIC CCK-8 IMMUNOREACTIVITY IN NORMAL AND CCl_4 INTOXICATED RABBITS ASSUMING DIETS AT DIFFERENT PROTEIN CONTENT

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INTRODUCTION

Over the past few years many authors suggested a new role for the gastrointestinal hormone CCK other than its known function as a stimulator of gall bladder contraction and pancreatic secretion (1,2). Both the finding of large concentration of CCK within the central nervous system of different species (3,4) and the modifications in feeding behaviour obtained after peripheral (5) or intraventricular injection (6) of CCK-like peptides indicated a potential function of CCK as satiety factor possibly modulating the satiety induced by serotonin. In addition we previously reported (7) changes in rat hypothalamic CCK-8 concentrations after acute or cronic intoxication with CCl_4, a drug able to induce liver necrosis and eventually an experimental liver cirrhosis (8).

In the present study we wanted to verify whether a different protein concentration in isocaloric diets could affect the CCK hypothalamic content of normal adult rabbits and possibly prevent the observed enhancement of hypothalamic CCK-8 in rabbits with hepatic damage induced by CCl_4 administration.

MATERIALS AND METHODS

Twenty adult male NZW rabbits after adaptation on a standard diet (STD), were weight sorted in three groups of five which were fed different diets:

1) high protein diet (HP) with a crude protein content (PC) of 27.7% (dry matter); 2) low protein diet (LP) with a PC of 4.8%; 3) STD diet (PC 18.3%) as a control.

Similarly another group of 12 adult NZW rabbits, chronically given intraperitoneal injection of CCl_4 (0,2 ml of 20% CCl_4 per 100 grams of weight per week for two months), after 8 days of standard diet were subdivided in three subgroups of four each receiving isocaloric diets at LP, STD and HP content respectively.

In both groups dietary manipulation were continued over 40 days, during which total food intake and body weight were monitored.

At the end of this period animals were sacrificed and the hypothalamus dissected and immediatily frozen.

Tissues were placed in distilled water (1:10 W/V), boiled for 20 minutes and then homogenised with a teflon tissue grinder. After centrifugation, supernatants were frozen until assayed.

CCK-8 immunoreactivity was measured with radioimmunoassay according to Straus et al. (9), synthetic CCK-8 (Squibb-Princeton N.Y. USA) was employed as standard and synthetic human I-125 Gastrin 17 (Becton-Dickinson, Milan) as a tracer. A COOH-terminal specific antibody (Rabbit B, a generous gift of Drs. R.S. Yalow and E. Straus, Bronx VAMC, N.Y.), which on a molar basis cross-reacts identically with all different CCK peptides, was employed.

Statistical analysis was performed with Student's test for un paired data.

RESULTS

In table I hypothalamic concentrations of CCK-8 immunoreactivity in normal rabbit fed diets at different protein content are reported. No significant difference was observed under various dietary regimens, although the body weight as well as the daily food intake resulted to be lower in animals treated by LP diets. (Fig. 1 e 2).

In table II hypothalamic CCK content in CCl_4-intoxicated rabbits is shown. In the owerall series CCK-8 immunoreactivity was significantly higher in these animals as compared to non-intoxicated controls (LP $p < 0.01$; STD $p < 0.025$; HP $p < 0.025$). However different protein supplements did not modify

the hypothalamic CCK-8 concentration even within this group of animals.

DISCUSSION

The result of this study indicate that various dietary supplement of protein in normal adult rabbits, although influencing food intake and body weight, do not modify the hypothalamic concentration of the satienty factor candidate cholecystokinin.

These data appaer to be in full accordance with those reported by Scheider and coll. (10) in rats and mice treated with various dietary manipulation. However the lack of relationship between hypothalamic CCK concentration and alterations in nutritional status does not necessarily mean that CCK does not act as a centralsatiety signal. First of all methods of analysis such as those employed in our study i. e. dissection of a cerebral region and radioimmunoassay of CCK immunoreactivity in tissue extracts might be not enough sensitive and refined to detect local neurophysiological perturbations. In addition, based on the greatly increased content of hypothalamic CCK we recently found in forcefed geese (Maleschi A., Nordio C. in press) we can speculate that only a condition of chronic hyperfagia be able to increase CCK neurotissutal concentrations as a result of a sustained stimulus to satiety. As to the significant echancement of hypothalamic CCK in CCl_4 treated rabbits it could well be the effect of the mild hepatic damage induced by the drug (steatosis and local necrosis at histology) similarly to what we observed in rats killed shortly after a single intraperitoneal injection of CCl_4 (7). In fact, due to a lower tolerance of the drug in rabbits, we were not able to induce in these animals an experimental liver cirrhosis with nodular regeneration as obtained after full-dose rat intoxication. In any event the fact that variations of protein intake, despite of the correcting effect of HP diet on the body weight and daily food intake (Fig. 1, 2) were not able to prevent such an increase of hypothalamic CCK, supports the hypothesis that there is not casual relationship between changes in CCK hypothalamic content and dietary modifications observed in these experimental conditions.

Therefore brain alterations concomitant to an experimental liver damage appear to be more likely related either to a direct neurotoxical effect of

CCl_4 or to a derangement of the CCK modulating dopaminergic system which can occur in presence of various degrees of hepatic failure (8).

TAB. I

Hypothalamic CCK-8 immunoreactivity (ng. of CCK-8 equivalents per gram of tissue) in normal rabbits assuming diets at different protein content (mean values ± S.D.)

Protein dietary content	CCK-8 I.R.	(n.)
4% (LP)	12.05 ± 2.36	(5)
18% (STD)	12.63 ± 4.34	(5)
28% (HP)	16.9 ± 3.74	(5)

TAB. II

Hypothalamic CCK-8 immunoreactivity (ng. of CCK-8 equivalents per gram of tissue) in rabbits intoxicated with carbon tetrachloride assuming diets at different protein content (mean values ± S.D)

Protein dietary content	CCK-8 I.R.	(n.)
4% (LP)	30.45 ± 10.4	(4)
18% (STD)	33.18 ± 17.55	(4)
28% (HP)	35.64 ± 17.28	(4)

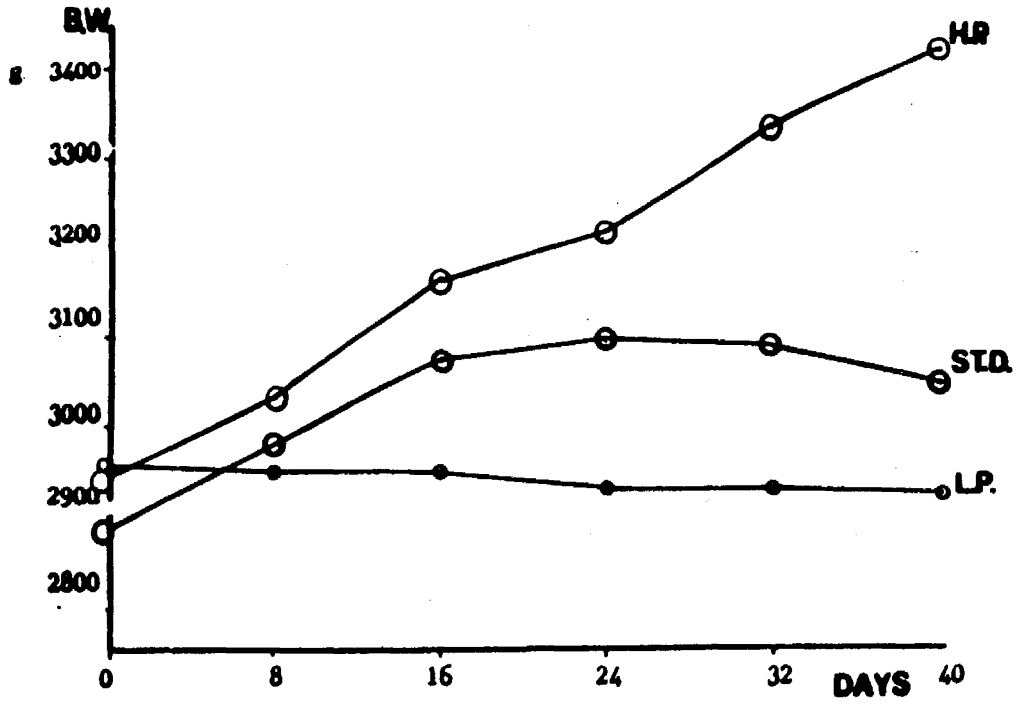

Fig 1 Behavior of the mean body weight of adult rabbits with CLD submitted to diets with different protein content: HP:high protein (27.7%), STD:standard (18.3%), LP:low protein (4.8%)

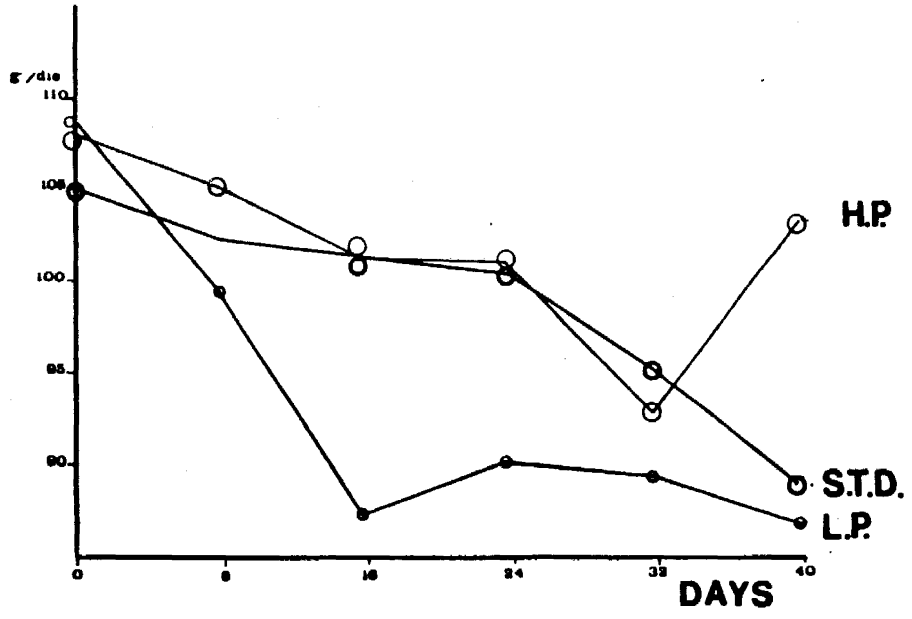

Fig.2 Average food intake of rabbits with CLD

REFERENCES

1) Gibbs J., Young R.C., Smith G.P. (1973), J. Comp. Physiol. Psycol., 84, 488.

2) Marley J.E., (1980), Life Sci. 27, 355.

3) Vanderhaegen J.J., (1975), Nature 257 604.

4) Straus E., Muller J.E., Choi H., Paronetto F. and Yalow R.S (1977), Proc. Natl. Acad. Sci, USA 74, 3033.

5) Autin J., Gibbs J., Holt J. et al. (1975), J. Comp. Physiol. Psycol. 89, 784.

6) Maddison S. (1977), Physiol. Behav. 19, 819.

7) Salerno F., Bonato C., Panerai A.E., Martini A., Maleschi A. (1983), Life Sci. 33, 377.

8) Zimmerman H.J., (1976), Exp. Production of Diseases, Springer Verlag, N.Y.

9) Straus E., and Yalow R.S (1978), Proc. Natl. Acad. Sci., 75, 486.

10) Schneider B.S., Monahan and Hirsch, (1979), J. Clin. Inv. 64, 1348.

SUMMARY

The hypothalamic concentration of CCK, a candidate for central control of food intake, was shown to be unaltered in rabbits fed diets at different protein content.

Significantly higher concentrations of hypothalamic CCK were found in rabbit with experimental liver damage (CCl_4 intoxication). Varying the dietary supplement did not prevent to any extent this enhancement of hypothalamic CCK content in CCl_4 intoxicated rabbits.

RIASSUNTO

E' stato dimostrato che la concentrazione ipotalamica di CCK, un probabile fattore di controllo centrale dell'assunzione di cibo, non risulta modificato in conigli alimentati con diete a differente contenuto proteico.

Sono stati altresì riscontrati più alti livelli di CCK ipotalamica in coni-

gli con danno epatico sperimentale da intossicazionz con CCl_4. Variazioni della somministrazione dietetica non interferiscono in alcun modo con i più alti livelli di concentrazione ipotalamica di CCK nei conigli intossicati con CCl_4.

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